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MICRORNA AND ENERGY METABOLISM IN GASTROINTESTINAL TUMORS

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“The value of a man should be seen in what he gives and not in what he is able to receive.”

Albert Einstein

ABSTRACT

MicroRNAs (miRNAs) contribute to cancer development and drug resistance via cellular biological processes, including metabolic pathways. Energy metabolism plays a significant role to maintain tumor proliferation in gastrointestinal (GI) cancers. However, the roles of miRNAs and energy metabolism in GI cancers are not fully understood. The studies presented in this thesis aim to provide further insights into the biological role of miRNAs, energy metabolism, and the interplay between miRNAs and energy metabolism in GI cancers, using gastrointestinal stromal tumor (GIST) and colon cancer as the models.

In **Paper I**, we explored the downstream target of *miR-125a-5p*-PTPN18 axis that contributes to imatinib resistance in GIST. We found that over-expression of *miR-125a-5p* and silencing of PTPN18 increased phosphorylated focal adhesion kinase (pFAK). FAK inhibitor 14, which blocks phosphorylation of Y397-FAK, enhances imatinib response in imatinib-resistant GIST cells. Furthermore, FAK inhibitor 14 could rescue the imatinib resistance mediated by overexpression of *miR-125a-5p*, suggesting that pFAK is the downstream target of the *miR-125a-5p*-PTPN18 axis.

In **Paper II**, we profiled the bioenergetic phenotype of imatinib-resistant GIST cells. We identified two major types of bioenergetics in imatinib-resistant GIST cell lines and clinical samples, i.e. highly metabolically active phenotype with higher glycolysis and oxidative phosphorylation (OXPHOS) and low OXPHOS types. Metabolic inhibitor assays revealed that imatinib-resistant GIST 882R cells (with highly metabolically active phenotype) were more sensitive to glycolysis inhibition than the parental GIST 882 cells, while imatinib-resistant GIST T1R cells (with low OXPHOS) were more resistant to OXPHOS inhibition than GIST T1. Our study demonstrates metabolic heterogeneity and diverse vulnerability of GIST cells to metabolic inhibitors, suggesting the potential of targeting energy metabolism for overcoming imatinib resistance in GIST.

In **Paper III**, we further explored the relationship between miRNA and imatinib treatment in GIST and the effect on OXPHOS. Using microarray and RT-qPCR, we identified *miR-483-3p* as one of the most downregulated miRNAs in imatinib-treated GISTs. Imatinib treatment resulted in downregulation of *miR-483-3p* and upregulation of OXPHOS in imatinib sensitive GIST cells. Modulation of *miR-483-3p* altered protein expression of mitochondrial respiratory Complex II, suggesting its involvement in OXPHOS regulation. This study reveals a potential role of *miR-483-3p* in imatinib-induced OXPHOS expression.

In **Paper IV**, we investigated the association of metformin treatment, an inhibitor of OXPHOS, with patient survival in colorectal cancer. We showed that metformin users were associated with 44% lower risk of mortality compared with nonusers. These findings suggest that metformin could be an adjunct to standard treatment of colorectal cancer.

Overall, this thesis work provides new insights into the role of miRNAs and energy metabolism in drug response and potential clinical use in GI cancers.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Wen-Kuan Huang**, Pinar Akçakaya, Anastasia Gangaev, Linkiat Lee, Katarina Zeljic. Praveensingh Hajeri, Erik Berglund, Mehran Ghaderi, Jan Åhlén, Robert Bränström, Catharina Larsson and Weng-Onn Lui. *miR-125a-5p* regulation increases phosphorylation of FAK that contributes to imatinib resistance in gastrointestinal stromal tumors.
Exp Cell Res 2018 Oct 1;371(1):287-296.

II. **Wen-Kuan Huang**, Jiwei Gao, Ziqing Chen, Hao Shi, Juan Yuan, Huanhuan L. Cui, Chun-Nan Yeh, Robert Bränström, Catharina Larsson, Shuijie Li and Weng-Onn Lui. Heterogeneity of metabolic vulnerability in imatinib-resistant gastrointestinal stromal tumor.
Cells 2020 May 26;9(6):1333.

III. **Wen-Kuan Huang**, Pinar Akçakaya, Katarina Zeljic, Anastasia Gangaev, Stefano Caramuta, Robert Bränström, Catharina Larsson and Weng-Onn Lui. Imatinib regulates *miR-483-3p* and oxidative phosphorylation in gastrointestinal stromal tumors.
Manuscript

IV. **Wen-Kuan Huang**, Shu-Hao Chang, Hung-Chih Hsu, Wen-Chi Chou, Tsai-Sheng Yang, Jen-Shi Chen, John Wen-Cheng Chang, Yung-Chang Lin, Chang-Fu Kuo and Lai-Chu See. Postdiagnostic metformin use and survival of patients with colorectal cancer: A nationwide cohort study.
Int J Cancer 2020 Oct 1;147(7):1904-1916.

OTHER PUBLICATIONS

1. Su PJ, Wu MH, Wang HM, Lee CL, **Huang WK**, Wu CE, Chang HK, Chao YK, Tseng CK, Chiu TK, Lin NM, Ye SR, Lee JY, Hsieh CH. Circulating Tumour Cells as an Independent Prognostic Factor in Patients with Advanced Oesophageal Squamous Cell Carcinoma Undergoing Chemoradiotherapy. *Sci Rep* 2016 Aug 17;6:31423.

2. Chen DY, See LC, Liu JR, Chuang CK, Pang ST, Hsieh IC, Wen MS, Chen TH, Lin YC, Liaw CC, Hsu CL, Chang JW, Kuo CF, **Huang WK**. Risk of Cardiovascular Ischemic Events After Surgical Castration and Gonadotropin-Releasing Hormone Agonist Therapy for Prostate Cancer: A Nationwide Cohort Study. *J Clin Oncol* 2017 Nov 10;35(32):3697-3705.

3. Kao WH, Kuo CF, Chou IJ, See LC, **Huang WK**, Chiou MJ, Zhang W, Doherty M, Wang CC, Hsu JT, Chen HH, Hong JH. Prostate-selective α antagonists increase fracture risk in prostate cancer patients with and without a history of androgen deprivation therapy: a nationwide population-based study. *Oncotarget* 2018 Jan 2;9(4):5263-5273.

4. Chou WC, Wu MH, Chang PH, Hsu HC, Chang GJ, **Huang WK**, Wu CE, Hsieh JC. A Prognostic Model Based on Circulating Tumour Cells is Useful for Identifying the Poorest Survival Outcome in Patients with Metastatic Colorectal Cancer. *Int J Biol Sci* 2018 Jan 12;14(2):137-146.

5. **Huang WK**, Juang YY, Chung CC, Chang SH, Chang JW, Lin YC, Wang HM, Chang HK, Chen JS, Tsai CS, Yu KH, Kuo CF, See LC. Timing and risk of mood disorders requiring psychotropics in long-term survivors of adult cancers: A nationwide cohort study. *J Affect Disord* 2018 Aug 15;236:80-87.

6. Hou CH, Lee JS, Lin KK, Chang SH, **Huang WK**, Kuo CF, See LC. Endophthalmitis Incidence of Cancer Patients After Cataract Surgery: A Nationwide Matched Cohort Study in Taiwan. *Am J Ophthalmol* 2019 Mar;199:246-254.

7. Chang PH, Wu MH, Liu SY, Wang HM, **Huang WK**, Liao CT, Yen TC, Ng SH, Chen JS, Lin YC, Lin HC, Hsieh JC. The Prognostic Roles of Pretreatment Circulating Tumor Cells, Circulating Cancer Stem-Like Cells, and Programmed Cell Death-1 Expression on Peripheral Lymphocytes in Patients with Initially Unresectable, Recurrent or Metastatic Head and Neck Cancer: An Exploratory Study of Three Biomarkers in One-time Blood Drawing. *Cancers* (Basel) 2019 Apr 15;11(4):540.

8. Chen DY, **Huang WK**, Chien-Chia Wu V, Chang WC, Chen JS, Chuang CK, Chu PH. Cardiovascular toxicity of immune checkpoint inhibitors in cancer patients: A review when cardiology meets immuno-oncology. *J Formos Med Assoc* 2019 Aug 20:S0929-6646(19)30408-5.

9. Lee CH, Hsieh JC, Wu TM, Yeh TS, Wang HM, Lin YC, Chen JS, Lee CL, **Huang WK**, Hung TM, Yen TT, Chan SC, Chou WC, Kuan FC, Hu CC, Chang PH. Baseline circulating stem-like cells predict survival in patients with metastatic breast Cancer. *BMC Cancer* 2019 Dec 2;19(1):1167.

CONTENTS

LIST OF ABBREVIATIONS

2-DG	2-deoxy-glucose
2-NBDG	2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose
3-BP	3-bromopyruvate
5-FU	5-fluorouracil
ABC	ATP-binding cassette
AKT	Protein kinase B
ADP	Adenosine diphosphate
AMP	Adenosine 5´-monophosphate
ATP	Adenosine triphosphate
AV	Annexin V
BCL2	B-cell lymphoma 2
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CDK	Cyclin-dependent kinase
CI	Confidence interval
COX	Cytochrome c oxidase complex
CRC	Colorectal cancer
DOG1	Discovered on GIST-1
ECAR	Extracellular acidification rate
EMT	Epithelial-mesenchymal transition
ETV-1	ETS translocation variant 1
FAK	Focal adhesion kinase
FDG-PET	^{18}F-fluorodeoxyglucose positron emission tomography
FH	Fumarate hydratase
GI	Gastrointestinal
GIST	Gastrointestinal stromal tumor
HIF-1α	Hypoxia-inducible factor-1α
HK	Hexokinase

IHC	Immunohistochemistry
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
LDH	Lactate dehydrogenase
MET	Hepatocyte growth factor receptor
miRNA	MicroRNA
mTOR	Mechanistic target of rapamycin kinase
NF1	Neurofibromatosis type 1
OCR	Oxygen consumption rate
ORR	Overall response rate
OXPHOS	Oxidative phosphorylation
PARP1	Poly [ADP-ribose] polymerase 1
PDGFRA	Platelet-derived growth factor receptor alpha
pFAK	Phosphorylated focal adhesion kinase
PGC1α	Proxisome proliferator-activated receptor gamma coactivator 1-alpha
PI	Propidium iodide
PI3K	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase
PKCθ	Protein kinase C theta
Pre-miRNA	Precursor miRNA
Pri-miRNA	Primary miRNA
PTPN18	Tyrosine-protein phosphatase non-receptor type 18
PTEN	Phosphatase and tensin homolog
RISC	RNA-induced silencing complex
ROS	Reactive oxygen species
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
SDH	Succinate dehydrogenase
shRNA	Short hairpin ribonucleic acid
siRNA	Small interfering ribonucleic acid
TCA	Tricarboxylic acid
TGF-β	Transforming growth factor beta

TFAM	Transcription factor A, mitochondrial
TKI	Tyrosine kinase inhibitors
UTR	Untranslated region
ZEB1	Zinc finger E-box binding protein 1

1 INTRODUCTION

MicroRNAs (miRNAs) are important regulators in gene expression networks, which can alter many biological processes. It is clear that miRNAs play important roles in gastrointestinal (GI) cancer development [1]. Energy metabolism has emerged to be potential therapeutic targets in cancer. This thesis has focused on miRNAs in regulating imatinib response, energy metabolism, and the potential therapeutics of metabolic inhibitor in GI cancers. Here gastrointestinal stromal tumor (GIST) was mainly used as the example of GI cancer.

1.1 GASTROINTESTINAL STROMAL TUMOR (GIST)

1.1.1 Clinical and molecular aspects

1.1.1.1 Epidemiology

GISTs are the commonest mesenchymal tumors of the GI tract. The incidence is rare, occurring at a rate of 0.5-2 cases per 100000 people annually [2]. In some regions, such as Shanxi - China and Czech republic, its annual incidence has been reported to be as low as 0.4 and 0.52 cases per 100000 people, respectively [3, 4]. In other parts, such as Korea [5], Taiwan [6] and Hong Kong [7], the annual incidence rates are as high as 1.9-2.2 cases per 100000 people. Registry data from 1992-2000 revealed an annual incidence of 0.68 per 100000 people [8]. However, GIST might be significantly under detected due to its asymptomatic nature and low malignancy. An autopsy study in elderly subjects reported 2 GISTs per 1000 cases [9], indicating that undetected GISTs with low malignant potential may be more common in the elderly. Such GISTs may occur more frequently than those of high malignant potential. Moreover, GIST incidence may change over time as awareness and diagnosis improve [10].

1.1.1.2 Pathology: historical review

GIST was first described by Mazur and Clark in 1983 as a wide spectrum of mesenchymal tumors that are ill defined by immunohistochemistry (IHC) and ultrastructural features [11]. Previously, several classifications have been used for GISTs, e.g. malignant fibrous histiocytomas, GI autonomic nerve tumors (GANTs), leiomyoblastoma, plexosarcomas, GI leiomyoma, sarcomas, or leiomyosarcoma. Given the presence of smooth muscle markers and the spindle cell morphology, GISTs were thought to be of smooth muscle origin. Later on, through electron microscopy and IHC analyses, they were labeled ‘stromal tumors’ given that their features differ from those of classic leiomyosarcoma. A key finding by Kindblom et al revealed that GIST cells are closely related to interstitial cells of Cajal based on IHC findings [12]. In the same year, Hirota and colleagues identified *KIT*-activating mutations

in most GISTs [13]. KIT (CD117) is a tyrosine kinase proto-oncogene that is highly expressed in interstitial cells of Cajal and GISTs. These findings lead to improved diagnosis of GIST and revealed the rationale for using tyrosine kinase inhibitors (TKIs) in GIST treatment.

1.1.1.3 Histopathology and molecular diagnosis

The size of GIST can vary from few millimeters to 35 cm and the median is 5-8 cm [9, 14]. GISTs are often surrounded by a pseudocapsule. At microscopic level, about 70% of GIST cells are spindle-shaped. However, about 20% of GISTs are comprised of epithelioid type or a mixture of spindle and epithelioid cells. Some GISTs exhibit pleomorphism. Central necrosis and cystic degeneration may exist in large GISTs. About 90-95% of GISTs are KIT positive, with a membranous, diffuse, mixed or focal staining pattern [15]. PKC-θ is abundant and constitutively phosphorylated in multiple GISTs. PKC-θ staining serves as a diagnostic marker of KIT-negative GISTs [16]. DOG1 (discovered on GIST1) has shown remarkably superior sensitivity (98%) and specificity for GIST, relative to KIT [17]. Notably, GISTs are generally negative for S-100 (95%) and desmin (98%) [18]. Combined IHC analysis of KIT and DOG1, or KIT and PKC-θ, may be more efficient for GIST diagnosis.

Activating mutations of the *KIT* and platelet-derived growth factor receptor alpha (PDGFRA) tyrosine kinases are the main factors driving the development of GIST [19]. These mutations are found in micro-GIST, suggesting that they are involved in early GIST development [20]. 70-75% of GISTs bear a single *KIT* mutation that affects juxtamembrane exon 11 (65%), extracellular domain exon 9 (10-20%), and kinase domains exon 13 (1%) or exon 17 (1%) [21]. 5-10% of GISTs have constitutively activating *PDGFRA* mutations. 80% of the *PDGFRA*-mutated tumors affect exon 18 (D842V mutation), while the others involve exon 12 (10-15%) or 14 (1-5%) [22, 23].

GISTs lacking *KIT* or *PDGFRA* mutations (10-20%) are termed “wild-type”, however, they have been shown to bear mutations affecting members of the succinate dehydrogenase (SDH) family, and sometimes in neurofibromatosis type 1 (*NF1*), or *BRAF* [24]. Thus, GISTs can be molecularly grouped into SDH-competent and SDH-deficient classes depending on SDH status. SDH-deficient GISTs tend to occur before 30 years of age, and predominantly affect females. They also present with multiple gastric lesions, plexiform features, epithelioid type, and lymph node metastases [25]. SDH-competent GISTs seldom exhibit the aforementioned features.

1.1.1.4 GIST-predisposing syndromes

Although risk factors for sporadic GIST are unknown, Carney's triad, Carney-Stratakis syndrome, and NF1, have been associated with GIST development. NF1 patients have higher risk of developing GIST relative to the general population [26]. The GISTs associated with NF1 are often multifocal, occur in small intestines, harbor *NF1* mutations, and have favorable prognosis. The Carney-Stratakis syndrome is rare and linked to germline mutations affecting subunits of the SDH complex, including SDHA, SDHB, SDHC, and SDHD [27]. These patients present with multifocal gastric GIST and paraganglioma at young age. Carney's triad is a rare non-hereditary syndrome that classically presents with multiple gastric GISTs (epithelioid type) in young women, extra-adrenal paraganglioma, and pulmonary chondroma. Promoter hypermethylation-mediated SDHC silencing is associated with GIST development in Carney's triad patients [28].

1.1.1.5 Clinical presentation

The median age at GIST diagnosis is 63-69 years and the disease afflicts men and women equally [8, 29]. The clinical presentation varies with location, tumor size, and growth conditions. GISTs are submucosal tumors, and may appear as ulcers and bleeding by invading through the mucosa. These tumors also invade via the serosa, spreading to nearby organs and leading to bowel perforation or intra-abdominal rupture. GIST symptoms include hematemesis, melena, fainting, and anemia, and may sometimes require emergency surgery. GISTs may also be indicated by palpable masses or pain, jaundice, early satiety, dysphagia, abdominal fullness, or intestinal obstruction. Although GISTs can affect any part of the GI tract, they most commonly affect the stomach (40-60%) and small intestines (30-40%) [8]. GISTs may also present as primary tumors in the omentum, mesentery or retroperitoneum [30]. At diagnosis, 20-25% of patients already have metastatic disease [31]. Metastases mainly affect the liver or abdominal cavity. Extra-abdominal metastases are rarely seen.

1.1.1.6 Prognosis and recurrence risk

The malignant potential of GIST varies widely, but even small GISTs (<2 cm diameter) may progress rapidly. Tumor size, location, and mitotic index are regarded as prognostic indicators [32, 33]. Proliferation index markers, like PCNA and Ki67, are also used to indicate prognosis [34]. Tumors arising in the stomach often have favorable outcomes, as opposed to those of the small bowel, colon, and rectum. Tumor rupture is positively related to the risk of local recurrence. Symptomatic cases may have poorer prognosis than asymptomatic ones [35].

1.1.1.7 Management of localized GIST

Surgery is the standard treatment for localized GIST and is aimed at en-bloc resection and intact capsule preservation to avoid tumor rupture or hemorrhage. A 1-2 cm macroscopic margin is considered optimal width for tumor-free margins [36]. However, lymph node dissection is not recommended since lymph node metastasis is uncommon in GIST. Surgery-associated morbidity may be substantial, and up-front surgery may cause extensive organ resection. Preoperative imatinib, a KIT and PDGFRA inhibitor, should be considered for tumor shrinkage, which may allow organ sparing. Prior to imatinib treatment, a tissue biopsy with mutational analysis is recommended. Although optimal duration is undefined, imatinib treatment for 6-12 months before surgery often achieves maximal tumor shrinkage. For patients at significant risk of recurrence, adjuvant imatinib is suggested for at least 3 years [37]. GISTs accompanied with mutations of *KIT* exon 11 may benefit from adjuvant imatinib, while those with *PDGFRA* D842V mutation or lacking *KIT* or *PDGFRA* mutation are unlikely to benefit. Patients with *KIT* exon 9 mutations are often given imatinib at up to 800 mg daily. Follow-up imaging at 3- or 4-month intervals is recommended for intermediate or high-risk tumors in the first 2 years after surgery since most recurrences occur in this window [37]. For those with low-risk tumors, a computerized tomography (CT) scan every 6 months for 5 years is advised. Surgical resection of primary GIST has been found to yield a 5-year survival rate of 48-70% [38, 39].

1.1.1.8 Management of advanced GIST

Approval for the use of imatinib in metastatic GISTs was granted in 2002 based on the positive outcomes from the European/Australasian (study 53) and American (study S0033) phase III studies [40, 41]. Metastatic or locally advanced GISTs are mainly treated with imatinib. For progressing disease treated with imatinib, sunitinib is used as the second-line drug [42]. For patients who fail to respond to imatinib and sunitinib, regorafenib is advised as third-line therapy [43]. Evidence suggests that metastasectomy may improve survival in some patients, especially where metastatic lesions cause infection, bleeding, or obstruction.

1.1.2 Imatinib resistance

The approval of imatinib led to the development of targeted therapy for targeting the impacts of mutations. Cumulative experience using imatinib and clinicopathological analyses has contributed to the elucidation of various responses based on the locations of mutations (Table 1). For example, the majority of patients with *KIT* exon 11 mutations benefit from

imatinib treatment. However, they generally develop imatinib resistance after about 8-24 months of disease stabilization or response [44].

Table 1: Distribution of *KIT* and *PDGFRA* mutations and their associations with imatinib response in GIST

Mutated gene	Exon (Percentage)	Imatinib response
KIT (70-75%)	Exon 11 (60-65%)	Well responded, while imatinib resistance typically occurs after 18 to 24 months of response or disease stabilization
	Exon 9 (10-12%)	Lower and shorter response compared to exon 11
	Exon 13 (3%)	Primary K642E mutation responded well
	Exon 17 (1%)	Non-responsive
PDGFRA (5-10%)	Exon 12 (1%)	Responded
	Exon 14 (1%)	Responded
	Exon 18, D842V (6%)	Non-responsive

The most common mechanism of resistance is reactivation of original tyrosine kinases via secondary point mutations (or secondary emergence due to selection pressure) (Figure 1). 46-67% of GIST patients with acquired imatinib resistance bear secondary mutations. Most secondary mutations are found in patients with primary *KIT* exon 11 mutations (73-86%), and only 21-33% of patients with *KIT* exon 9 primary mutations develop secondary mutations [45, 46]. The commonest secondary *KIT* mutation in GIST patients with acquired resistance is amino acid substitutions clustered on the ATP-binding pocket site (exon 13 or 14) of KIT, such as V654A and T670I. Secondary mutations also affect the activation loop (exon 17 or 18), e.g. C809G, D816G/H, D820A/E/G/N/V/Y, N822H/K/T/Y, and Y823D, of the same allele as the primary mutation [44, 45, 47]. Such mutations occlude the access of imatinib via steric hindrance or loss of crucial hydrogen bonds, thus conferring acquired imatinib resistance. *KIT* amplification is also seen in imatinib-resistant patients [48]. These findings show that acquired resistance to imatinib involves original tyrosine kinases, but not activation of additional signaling pathways, highlighting unique oncogene addiction by these tumors.

Imatinib resistance may also result from kinase switching, where alternative kinases, independent of the original one are activated. In chronic myeloid leukemia, imatinib resistance has been shown to rely on upregulation of LYN and HCK. In GIST, activation of focal adhesion kinase (FAK) [49], AXL [50], SRC/integrin [51], and hepatocyte growth factor receptor (MET) [52] are also thought to mediate acquired imatinib resistance. Additionally, fibroblast growth factor receptor 3 (FGFR3) can restore KIT phosphorylation and promote imatinib resistance via MAPK signaling activation [53]. Thus, combining imatinib with inhibitors of these kinases may enhance anti-tumor effects.

Alternatively, some imatinib-resistant tumors can lose KIT expression by loss of heterozygosity at the *KIT* locus, and undergo a phenotypic alteration to an undifferentiated phenotype [54]. Additionally, drug influx/efflux mediators also influence imatinib resistance. Upregulation of multidrug resistance 1 (MDR1), an ATP-binding cassette (ABC) transporter, is known to reduce intracellular levels of chemotherapy agents by extruding them [55].

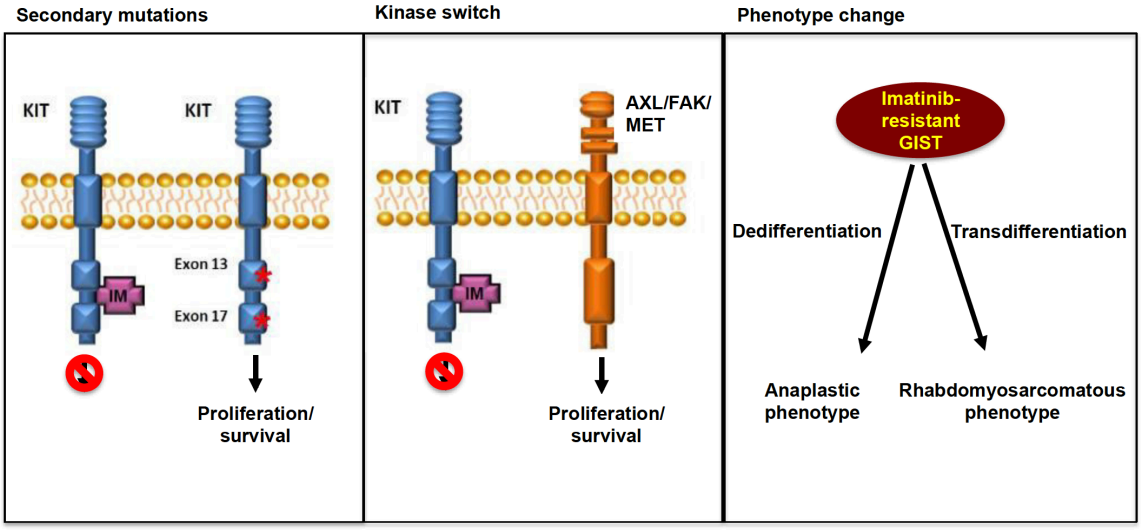

Figure 1: Mechanisms involved in acquired imatinib resistance in GIST. Acquired secondary *KIT* mutations that hinder imatinib binding are the commonest resistance mechanism. Other mechanisms include kinase switching from KIT to other receptor tyrosine kinases, and phenotypic alterations in which tumor cells lose KIT expression and undergo phenotypic changes to dedifferentiated phenotype (Courtesy of Pinar Akcakaya).

1.1.3 Emerging tyrosine kinase inhibitors (TKIs)

The limited efficacy seen with 2nd and 3rd line TKIs indicates that there is an urgent need to overcome TKIs resistance in GIST. To this end, the targeted drugs, avapritinib (BLU-285) and ripretinib (DCC-2618) are in international phase III studies. Ripretinib is a type II kinase inhibitor with a "switch-control" function that forces the activation loop into an inactive conformation by restoring juxtamembrane domain inhibitory switch [56]. Recently preliminary findings of the phase III study (Invictus) was released, which revealed a median progression-free survival of 6.3 vs 1 month for ripretinib vs. placebo in heavily-treated patients (>3 lines of treatment) [57]. However, the overall survival benefits have not been released. Notably, the objective response rate of ripretinib was 9.4%, which is similar to sunitinib or regorafenib. A summary of recent advances in targeted therapies for GIST treatment is shown in Table 2.

Table 2: Selected targeted drugs overcoming imatinib resistance or immunotherapy in GIST

Drug (Manufacturer)	Mechanism	Study phase	Anti-tumor efficacy

Avapritinib (Blueprint)	A selective inhibitor of KIT and PDGFRA activation loop mutants, including *KIT* exon 17 and *PDGFRA* exon 18 D842V	I	In the updated data of phase I NAVIGATOR trial (n=237), the overall response rate (ORR) for patients with more than 4 lines treatment failure (n=111) was 22%. 52 patients (47%) had stable disease with the median duration of response (mDOR) of 10.2 months. Most adverse events (AE) were grade 1-2. Of them, memory impairment (29%) is the most common cognitive AE. The unprecedented activity in ORR and mDOR suggests avapritinib having a potential to change the treatment paradigm. However, recently T670I gatekeeper mutation was evidenced to confer avapritinib resistance [58].
Ripretinib (Deciphera)	An inhibitor of full spectrum of mutant KIT and PDGFRA kinases with "switch-control" ability	III	In the phase III INVICTUS trial, ripretinib (DCC-2618) demonstrated a dramatic improvement in progression-free survival (PFS) compared with placebo (6.3 months vs. 1 month) [57].
Cabozantinib (Exelixis)	A TKI targeting KIT/ MET/AXL/VEGFR	II	30 of the 50 patients (60%) had progression-free disease at week 12. 7 patients (14%) achieved a partial response and 33 (66%) had stable disease [59].

Although sunitinib and sorafenib are known to be active against GIST after imatinib failure, their clinical efficacy is limited by lower tumor response rate and shorter progression-free survival relative to imatinib, indicating that KIT-mediated resistance remains a major hurdle to effective anti-tumor therapy. Emerging anti-KIT agents, such as ripretinib or avapritinib, may improve GIST prognosis after imatinib failure. However, regorafenib, a potent inhibitor of exon 17/18 mutations, has not exhibited satisfactory results in clinical trials. Thus, the signaling pathways with potential alternative therapeutic targets to overcome drug resistance remain elusive.

1.2 MICRORNA IN CANCER

1.2.1 Biogenesis

miRNAs are a class of multifunctional short non-coding RNAs that modulate the stability and/or translation of target mRNAs. Through sequence-specific interaction with the 3'-untranslated region (UTR) of target mRNA, miRNAs suppress gene expression by triggering transcript degradation or inhibition of protein translation. More than 2600 mature miRNAs have been identified in various human tissues and cells based on miRBase release

22.1 (October 2018) [60]. miRNA biogenesis begins with RNA polymerase II mediated synthesis of primary miRNAs (pri-miRNAs), which are about 1 kb long. Pri-miRNAs are then processed in the nucleus by a microprocessor complex comprised of Drosha and DiGeorge syndrome critical region 8 (DGCR8) into precursor miRNAs (pre-miRNAs) (50-110 bp long) with stem-loop-stem structures. The pre-miRNA is then transported from the nucleus into the cytoplasm by exportin-5, a pre-miRNA-specific export carrier on the nuclear membrane. The terminal loop of pre-miRNAs is removed by RNase III Dicer and TAR RNA binding protein 2 (TARBP2) complex, generating double-stranded miRNAs that are about 18-26 bp long. The mature miRNA is incorporated into RNA-induced silencing complex (RISC), a ribonucleoprotein complex. The miR-RISC complex enables base-pairing interaction between a miRNA and its target RNA's binding site, mediated by Argonaute (AGO) and various cofactors. This interaction may cause translational repression or mRNA degradation via endonucleolytic cleavage or deadenylation of target mRNA. The precise mechanism may depend on the base-pairing complementarity between the miRNA and target mRNA [61].

1.2.2 Aberrant miRNA expression in cancer

miRNAs were first implicated in cancer when Calin et al. found that *miR-15* and *miR-16* hosted in chromosome 13q14, are downregulated in about 68% of patients with chronic lymphocytic leukemia [62]. Subsequent studies revealed that miRNA expression signatures can accurately classify cancer types [63], including hepatocellular carcinoma [64], colon cancer [65], and bladder cancer [66]. These studies offered a rationale for miRNA as diagnostic tools.

MiRNA dysregulation may occur via chromosomal abnormalities, transcriptional changes, epigenetic modifications, and defects in the miRNA synthesis machinery. Amplification or deletion of genomic regions containing miRNA genes may contribute to abnormal miRNA expressions. For example, *miR-143* and *miR-145* in the 5q33 region are often deleted in lung cancer, causing their downregulation [67]. In contrast, the 13q31 region harboring the *mir-17~92* cluster gene is often overexpressed in lung cancers [68]. MiRNA transcription may be controlled by various transcription factors. The oncogenic *mir-17~92* cluster is activated by c-Myc or E2F [69, 70]. Wang et al. found a double-negative feedback loop between c-Myc and *miR-122,* a tumor suppressor in hepatocellular carcinoma [71]. p53 induces *miR-34a* expression via direct binding to its promoter region, promoting cell apoptosis [72]. miRNAs are susceptible to epigenetic modulation, including global hypomethylation, CpG island hypermethylation, histone modifications, and nucleosomal remodeling. The aberrant fusion protein AML1/ETO, most commonly found in acute myeloid leukemia, directly

suppresses *miR-223* expression via CpG island hypermethylation [73]. *MiR-127,* which targets the BCL6 proto-oncogene, is embedded in a CpG island and is subjected to epigenetic silencing in cancer [74]. In addition, mutation or deregulation of miRNA processing factors can also alter miRNA expression. For example, DGCR8 and Drosha mutations alter the expression of *let-7a* and *miR-200* family members in Wilm's tumors [75].

1.2.3 Regulation of biological pathways by miRNA

The role of miRNAs in tumorigenesis has been extensively studied. Aberrant cell cycle activation in cancer is attributed to cyclins, cyclin-dependent kinases (CDKs) and their inhibitors, which are widely modulated by miRNAs. *MiR-15a* and *miR-16-1* cause cell cycle arrest at G1 by targeting cyclin D, cyclin E, and CDK6 [76, 77]. In breast cancer, cyclin D1, which is regulated by *miR-17/20*, controls cell cycle progression [78]. In gastric cancer, *miR-221/222* directly suppresses the CDK inhibitors $p27^{KIP1}$ and $p57^{KIP2}$, thereby facilitating G1/S phase transition [79]. Modulation of p27 KIP1 by *miR-221/222* has also been found in glioblastoma [80], prostate cancer [81], and thyroid cancer [82], implicating *miR-221/222* mediated regulation of $p27^{KIP1}$ as a common oncogenic pathway in several cancer types. Multiple miRNAs influence resistance to cell death. Downregulation of *miR-15a* and *miR-16-1* promotes cancer cell proliferation by elevating levels of B-cell lymphoma 2 (BCL2), an anti-apoptotic factor [83, 84]. Multiple p53-regulated miRNAs have been implicated in a feedback loop with p53. *MiR-192*, *miR-194* and *miR-215*, which are activated by p53, directly suppress Mdm2 expression that prevents p53 degradation in multiple myeloma [85]. In hypoxic conditions, the *miR-17~92* cluster is downregulated by p53, leading to apoptosis [86]. Thus, p53 and associated miRNAs form a network that mediates resistance to cell death.

MiRNAs may also regulate epithelial-mesenchymal transition (EMT) by targeting transforming growth factor beta (TGF-β) or other EMT-associated pathways. TGF-β/SMAD4 signaling activates *miR-155*, which targets RhoA GTPase, an important modulator of cell migration and invasion [87]. The transcription factors ZEB1/2 are well known EMT regulators, and together with the *miR-200* family, form a double-negative feedback loop to regulate cancer cell invasion [88]. Several miRNAs, including *miR-203*, *miR-9*, and *miR-212* influence cancer metastases by modulating key transcription factors, including SNAI2, c-Myc, and MnSOD [89-91]. MiRNAs promote angiogenesis, particularly in hypoxic conditions. *miR-210* is the most consistently induced miRNA under hypoxia. *miR-210* facilitates angiogenesis by targeting the receptor tyrosine kinase ligand ephrin-A3, which is an antiangiogenic factor, thereby enhancing VEGF receptor-2 expression [92, 93]. Thus, miRNAs may exhibit tumor suppressive or oncogenic function by modulating various pathways.

1.2.4 Clinical impacts of miRNAs

1.2.4.1 Diagnosis

The miRNA expression levels may identify tumor origins, suggesting the presence of cancer tissue-specific miRNA expression profiles [94]. Multiple studies have suggested miRNAs as diagnostic biomarkers for specific cancers. For example, *miR-192*, *miR-215* and *miR-148a* are tumor suppressors in colorectal cancer (CRC), and their downregulation is used as diagnostic biomarkers [95]. Various circulating miRNAs, including *miR-215*, *miR-299-5p* and *miR-411* are breast cancer biomarkers [96]. *miR-21*, *miR-126*, *miR-486-5p*, and *miR-210* have exhibited remarkable diagnostic efficiency in non-small cell lung cancer [97]. *miR-205* and *miR-21* overexpression in pancreatic ductal adenocarcinoma precedes pancreatic ducts phenotypic changes, suggesting their potential value in early detection [98]. Furthermore, miRNA profiles can identify cancer subtypes, including basal and luminal breast cancers [99]. However, the clinical use of miRNAs as diagnostic biomarkers still requires comprehensive validation in a large cohort.

1.2.4.2 Prognosis

Multiple studies have highlighted miRNAs as cancer prognostic biomarkers. Calin et al. identified a specific miRNA signature associated with progression and prognosis of chronic lymphocytic leukemia [100]. *miR-155* overexpression and *let-7a* downregulation are associated with poor prognosis in lung cancer [101]. *miR-210* upregulation has been linked to poor clinical outcomes in triple negative breast cancer [102, 103]. However, the use of miRNAs as prognostic markers in clinical practice is constrained by poor understanding of whether they are reproducible and disease specific. Further prospective studies are warranted to validate their prognostic power.

1.2.4.3 Potential therapeutic application in pre-clinical models

MiRNA-based therapies include miRNA mimics and inhibitors (anti-miRs). MiRNA mimics are small chemically modified RNA molecules matching the corresponding endogenous mature miRNA. These agents are designed to functionally replace lost miRNA expression. In contrast, anti-miRs are single-stranded antisense oligonucleotides with chemical modifications that block miRNA function. Kasinski et al. showed that nanodelivery of *miR-34* and *let-7* suppressed tumor growth in a murine model of non-small cell lung cancer by suppressing expression of the tumor promoters Lin28b, c-Met and Myc [104]. Two independent studies found that *miR-520e* and *miR-375* suppress liver cancer cell growth in xenograft models [105, 106]. Inhibition of oncogenic *miR-21* expression combined with

gemcitabine promotes apoptosis in a mouse model of pancreatic ductal adenocarcinoma [107]. Although their therapeutic use is attractive, few miRNA mimics or inhibitors have entered clinical development. Their clinical use is constrained by lack of effective, and safe delivery systems [108].

1.2.5 miRNA in drug resistance

miRNA may play different roles in regulating drug resistance in different cellular contexts. For example, *let-7b* enhances tamoxifen sensitivity by targeting estrogen receptor alpha signaling in breast cancer [109]. In contrast, in ovarian cancer, *let-7b* reduces taxane sensitivity via downregulating insulin like growth factor 2 mRNA binding protein 1 (IMP-1), which is an RNA binding protein stabilizing a number of target genes [110]. MiRNAs can regulate drug resistance through different biological processes such as drug metabolism. For example, downregulation of CYP3A4 by *miR-27b* was found to cause cyclophosphamide resistance in pancreatic cancer cells [111]. MiRNAs can regulate drug transporters to affect chemotherapy sensitivity. *miR-298*, *miR-145* and *miR-451* regulate doxorubicin sensitivity by targeting the P-glycoprotein drug transporter in breast cancers [112]. Furthermore, miRNAs can also target DNA repair genes to alter the chemotherapy response. For example, MutS Homolog 2 (MSH2) and MSH6 were downregulated by *miR-21* to confer 5-fluorouracil resistance [113]. MiRNAs can enhance drug resistance by regulating key genes involved in cell proliferation and apoptosis-related signaling pathways. For example, PTEN/AKT pathway is a target of *miR-21* or *miR-214* to induce chemotherapy resistance in cholangiocarcinoma or ovarian cancer, respectively [114, 115]. Additionally, miRNAs can regulate key genes associated with apoptosis. Bcl-2 is an anti-apoptotic protein in the Bcl-2 gene family. Inhibition of *miR-15a/16* upregulates BCL-2 expression and promotes tamoxifen resistance in breast tumor cells [116]. Of note, miRNAs play roles in targeted drug resistance by modulating genes associated with EMT. *MiR*-200c regulates trastuzumab by targeting Zinc finger protein 217 (ZNF217) and Zinc finger E-box binding protein 1 (ZEB1) in breast cancer [117]. TGFβ-*miR-200*-mitogen-inducible gene 6 (MIG6) signaling was found to orchestrate the EMT-associated kinase switch that induces resistance to gefitinib [118]. Since miRNAs can effectively regulate chemotherapy response in tumor cells, the use of chemotherapy in combination with modulation of miRNA is an optimal strategy to achieve a better response. Wu et al. found that *miR-27b* could enhance the anticancer effect by activating p53 and inhibiting CYP1B1, demonstrating that the synergistic effect in combination of miRNA in cancer treatment [119]. Furthermore, several studies showed that co-encapsulation of miRNAs with small molecule drugs in a nanocarrier, such as *miR*-205 and gemcitabine for pancreatic cancer treatment [120].

Further investigations are warranted to evaluate the combinatorial effect of miRNA therapeutics and anti-cancer treatment for overcoming drug resistance.

1.2.6 miRNA in energy metabolism

Bioenergetic reprogramming is an emerging hallmark of cancer cells [121]. Several studies found that miRNAs play crucial roles in modulating energy metabolism in cancer cells. For example, *miR-155* has been reported to enhance glycolysis through activation of hexokinase 2 (HK2), an enzyme for the irreversible first step of glycolysis [122]. Tricarboxylic acid (TCA) cycle can also be regulated by *miR-155*. Silencing of *miR-155* leads to reduced expression of two thiamine transporters, SLC19A2 and SLC25A19, with a concordant reduction in thiamine [123]. *MiR-378-3p* can suppress TCA cycle by direct targeting of ERRγ and GA-binding protein alpha chain (GABPA) [124]. cMyc suppresses *miR-23a/b* and thereby leads to upregulation of mitochondrial glutaminase, enhancing glutaminolysis, which is also a common metabolic reprograming seen in cancer cells [125]. Upregulation of hypoxia-inducible factor-1α (HIF-1α) under hypoxia suppresses the expression of *miR-199a-5p*, which can directly target the 3'-UTR of HK2 [126]. Thus, *miR-199a-5p*/HK2 reprogramming enhances glycolysis in liver cancer cells, promoting cell proliferation and tumorigenesis. HK2 can also be targeted by *miR-143* in head and neck squamous cell carcinoma, breast cancer, and colon cancer [122, 127, 128]. On the other hand, miRNAs can regulate transcripts of TCA cycle or electronic transport chain in cancer cells. For instance, *miR-183* downregulates isocitrate dehydrogenase 2 (IDH2) that leads to induction of HIF-1α in glioma cells [129]. SDH targeted by *miR-210* is able to activate HIF-1 and the consequences on cell metabolism and survival [130]. The nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) signaling pathway suppresses the expression of *miR-181c*, which can directly downregulate cytochrome c oxidase complex 1 (COX1) [131]. COX4 is found to be the direct target of *miR-338* [132]. Altogether, these findings showed that miRNAs can alter energy metabolism processes by regulating metabolism-associated genes.

1.3 ROLE OF MIRNAS IN GIST

1.3.1 Aberrant miRNA expression in GIST

miRNA profiling of GIST was first reported in 2008, which was found distinct from other sarcoma types [133]. Notably, expression of *mir-221~222* and *mir-17~92* clusters was found to be downregulated relative to other sarcomas. KIT and ETS translocation variant 1

(ETV-1), the key oncogenes in GIST, are direct targets of the *mir-221~222* and *mir-17~92* clusters, respectively [134], suggesting that these miRNAs may influence tumorigenesis. Moreover, miRNA profiles can distinguish between *KIT*-mutated vs *PDGFRA*-mutated [135], high-risk vs low-risk [136, 137], imatinib-sensitive vs resistant [138], and metastatic vs. non-metastatic GISTs [138].

1.3.2 miRNA involvement in GIST progression

Multiple dysregulated miRNAs are associated with apoptosis and cell proliferation in GIST. *MiR-222/221* induces apoptosis via KIT/AKT signaling [139, 140]. Loss of 14q is common in GIST. *MiR-494*, a miRNA located in this region, functionally suppresses GIST growth by targeting KIT and survivin [141, 142]. Downregulation of *miR-133b* and overexpression of fascin-1 may contribute to GIST aggressiveness [137]. *MiR-17/20a* directly suppresses ETV-1, inducing apoptosis [134]. Some miRNAs have been reported to regulate imatinib response. *MiR-518a-5p* downregulation enhances Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing beta polypeptide (PIK3C2A) expression, leading to imatinib resistance [143]. PTPN18 suppression via *miR-125a* upregulation causes imatinib resistance [138]. Thus, in GIST, miRNA dyregulation mediates invasion, proliferation, apoptosis or imatinib resistance via key molecular pathways.

1.3.3 Potential role of miRNAs in prognostic prediction and treatment

Upregulation of *miR-196a* is associated with high-risk grade, metastasis and poor prognosis [144], while downregulation of *miR-186* associates with metastatic recurrence and poor survival in GIST [145]. High *miR-1915* levels associate with improved survival in GIST patients [138]. These reports indicate a potential role for miRNAs as prognostic markers. However, further studies to validate these miRNAs in combination with known prognostic factors, including tumor location, size, and mitotic index, are necessary. Application of miRNAs in GIST treatment, especially in rescuing imatinib resistance, is a major clinical challenge. However, in pre-clinical studies, *miR-218* loaded nanoparticles have been demonstrated to suppress GIST growth, and overexpression of *miR-218* enhances imatinib response in GIST cells [146]. Chemically modified *miR-221/222*, with altered nucleotides in the seed region, are effective suppressors of KIT expression, and may therefore help to overcome imatinib resistance [134]. To date, miRNAs have not entered clinical trials for GIST treatment and their clinical value remains to be determined.

1.4 ENERGY METABOLISM

1.4.1 Energy metabolism in cancer

All cells need catabolites to maintain energy metabolism for cell growth and division. It has long been recognized that tumor cells require higher demand of energy metabolism. The central axis of energy metabolism consists of glycolysis and TCA cycle coupling with OXPHOS. Glucose can be catabolized through glycolysis with generation of lactate and 2 moles of adenosine triphosphate (ATP) per mole of glucose. Further metabolism of pyruvate from glycolysis in the mitochondria can generate 36 moles of ATP per mole of glucose via the TCA-OXPHOS cycle, which is 18 times more than the amount from glycolysis [147]. Although cells use both pathways, one pathway frequently dominates to maintain bioenergetic requirement.

Approximately one century ago, Warburg discovered a bioenergetic difference between cancer and normal cells, in which cancer cells tend to utilize glycolysis and produce lactate, while normal cells use TCA-OXPHOS cycle to generate ATP and release CO_2 (Figure 2) [148]. Several hypotheses explain why the "Warburg effect" preferentially happens in tumor cells. First, OXPHOS is not effective for cell proliferation especially when the resources are scarce in the microenvironment. Second, the glycolytic switch in tumor cells not only produces effective ATP supply but also provides glycolytic intermediates to supplement biosynthetic pathways, which are responsible for synthesis of the biomacromolecules such as nucleotides and amino acids required for proliferating tumor cells. Excess lactate, typically a metabolic waste product derived from glycolysis, is utilized for ATP production and biomacromolecules synthesis [149]. Stromal cells and neighboring tumor cells can utilize lactate derived from glycolysis-dominant tumor cells to produce pyruvate, which in turn can refuel tumor cells. Anaerobic tumor cells, aerobic nontransformed stromal cells and neighboring tumor cells thereby form the cancer symbiosis relationship (Figure 3) [150]. Furthermore, glutamine is also utilized for synthesizing macromolecules, such as production of nonessential amino acids, lipids, and nucleotides, in tumor cells.

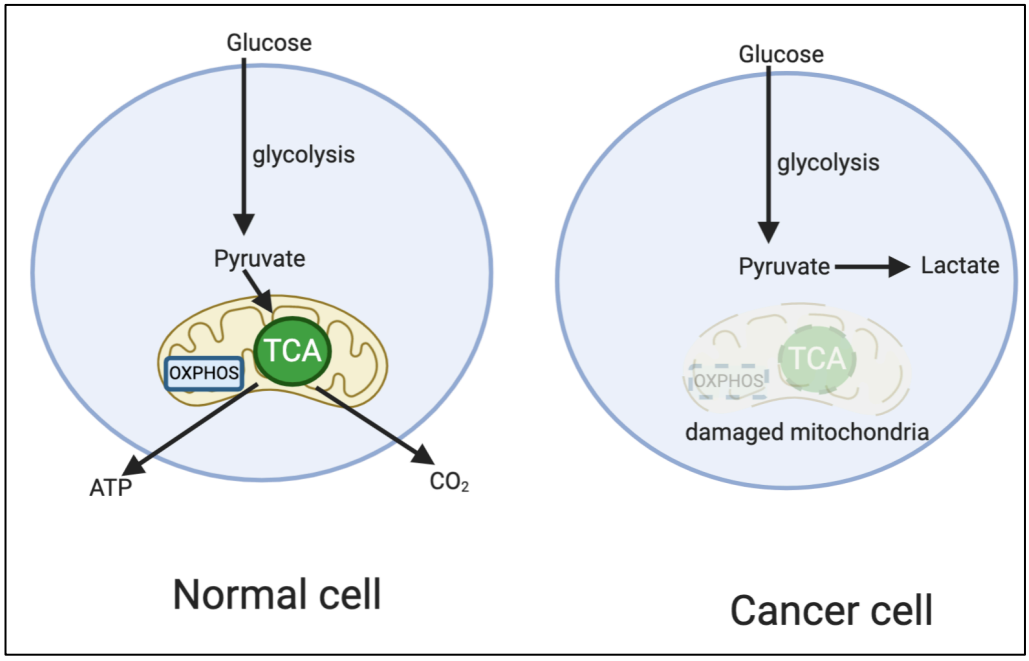

Figure 2. Energy metabolisms from classical Warburg effect. Cancer cells utilize glycolysis, while normal cells use the TCA cycle for ATP production.

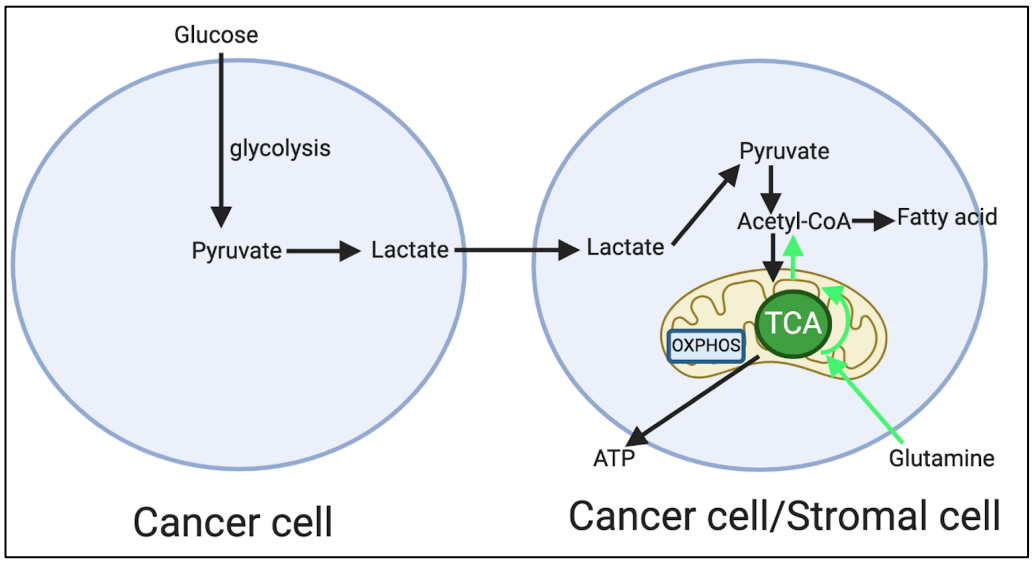

Figure 3. Cancer cell symbiosis model. The lactate can be utilized by neighboring tumor cells or stromal cells for energy refuel or macromolecules biosynthesis.

The Warburg metabolism forms the basis of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) imaging. At the first step of glycolytic process, glucose is converted to glucose-6-phosphate (G-6-P) by HK enzymes. There are four HK isoenzymes in mammalian cells. Among them, HK2 is predominantly expressed in tumor cells. 2-^{18}F-fluoro-2-deoxy-D-glucose (FDG) can be phosphorylated by mitochondrial bound HK2 to FDG-6-

phosphate, which is unable to be metabolized further and thereby accumulate in the tumor cells with higher level than that in normal cells.

Otto Warburg's hypothesis led to the widely held misconception that cancer cells rely on glycolysis as their major source of ATP [151]. Although Warburg initially claimed that the effect of glycolysis taken by tumor cells even in the presence of ambient oxygen is due to impaired mitochondria, subsequent studies found that the mitochondrial function in tumor cells is intact [147]. To date we know that it is not impaired mitochondria but several gene mutations involving in energy metabolism contribute the shift from OXPHOS to glycolysis [152]. For example, several oncogenes, such as HIF-1α, MYC, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), AKT and mechanistic target of rapamycin kinase (mTOR), can upregulate transcription of genes encoding glycolysis-associated proteins. Wild type *TP53*, a master tumor suppressor gene, suppresses glycolysis, while mutant p53 is known to increase glycolysis in tumor cells. Furthermore, reducing ATP production by inhibition of pyruvate kinase fails to restrain tumorigenesis, suggesting that using glycolysis to supply ATP is not the major function to support tumor growth [153]. Several studies have revealed that majority of tumor cells also have the capacity to produce ATP through OXPHOS [154]. Moreover, tumorigenesis and tumor cell proliferation have been shown to rely on mitochondrial metabolism [155]. Mutations in enzymes involved in TCA cycle, such as SDH and fumarate hydratase (FH), might be exceptions. However, cancer cells with mutations in FH or SDH still metabolically rewire to mitochondrial metabolism to provide TCA cycle intermediates for further macromolecules biosynthesis [149, 156, 157].

In addition to pyruvate derived from glycolysis, fatty acids and amino acids can supply substrates to the TCA cycle to maintain oxidative ATP production in cancer cells. Fatty acids are catalyzed to acetyl-CoA through β-oxidation in the mitochondria and generate the reducing NADH and FADH2 co-factors, which transfer electrons to oxygen for mitochondrial ATP production. The amino acid glutamine metabolism can generate α-ketoglutarate to supplement the TCA cycle through glutaminolysis [158]. Furthermore, elevation of circulating branched chain amino acids isoleucine, valine, and leucine can be catabolised to acetyl-CoA and other TCA cycle intermediates [159]. The metabolic flexibility allowing multiple inputs into the TCA cycle that provides fuels to cancer cells, which allow them to respond to changing microenvironment such as hypoxia or nutrition depletion.

Tumor cells bioenergetically adapt to stress microenvironment by sensing the ATP/adenosine diphosphate (ADP) ratio. In nutrition-deficient environment, tumor cells decrease

the demand of ATP to increase the available ATP, thereby sustaining the ATP/ADP ratio. If diminishing ATP demand is not able to maintain the ATP/ADP ratio, the rise in ADP activates adenylate kinase, which converts two ADP molecules into 1 molecule adenosine 5´-monophosphate (AMP) and 1 molecule ATP [160]. The rise in AMP during nutrient deprivation triggers the activation of AMP kinase (AMPK), facilitating catabolic pathways like fatty acid oxidation, glycolysis and pentose phosphate pathway (PPP), to stimulate ATP production. For example, hypoxic or Ras-driven cancer cells consume lipids to support ATP production under starvation condition [161]. Therefore cancer cells maintain their ATP/ADP ratio to sustain nutrient- and oxygen-deficeint stress.

Reduced amino acids or oxygen suppress the activity of mTOR, which is an anabolic kinase driving the energetically demanding growth of tumor cells [162]. Furthermore, inhibition of mTOR increases autophagy flux, which can engulf damaged organelle and cytosolic macromolecules and deliver them to lysosomes for degradation. In non–small-cell lung cancer (NSCLC) cells harboring oncogenic *KRAS*- or *BRAF* mutations, autophagy provides glutamine supply to maintain oxidative metabolism [163]. From the 1980s, scientists demonstrated that dysregulation of oncogenes and tumor suppressors were associated with tumor formation, leading to the identification of mutations as a cause of cancer [164]. Subsequent studies with extensive investigation using next-generation sequencing and omics-approaches discovered thousands of mutations for different cancer types. Therefore, cancer has been recognized as a complex genetic disease caused by the activation of oncogenes or loss of tumor suppressor genes, facilitating deregulation of cell proliferation and apoptosis. Extensive omics approaches have tried to identify the way to cure cancer by targeting the mutations driving the tumor growth. Furthermore, the considerable genetic and histological heterogeneity of tumors seems to converge into the common finite set of pathways to support fundamental functions such as catabolism, anabolism, and redox balance. This raises a question whether cancer metabolism itself can be targeted to control cancer growth. Finding a cure for cancer by regulating cancer metabolism have become a promising strategy [164].

For targeting metabolism a few things have to be considered for the anti-metabolism drug development. First, proliferating cells, such as immune cells and stem cells, can reprogram their metabolic pattern similar to cancer cells [165, 166], which may limit the therapeutic feasibility of inhibiting certain metabolic enzymes. Additionally, the blockade of certain metabolic pathway may not be tolerated due to interference with the physiological functions of the normal tissues. Indeed, several excellent examples demonstrate the adequate

therapeutic window for targeting the reprogramming metabolic pathway, such as nucleotides synthesis by antifolates (methotrexate, pemetrexed). Second, cancer cells can utilize metabolic plasticity to reprogram their metabolic profile during metastasis and drug stress. Thus, it is conceivable that cancer cells could resist to inhibition of a particular metabolic pathway by upregulating compensatory pathways. Therefore, a reasonable therapeutic strategy should target several metabolic pathways simultaneously or target a particular metabolic pathway in combination with therapies to suppress compensatory signaling pathways.

1.4.2 Energy metabolism in GIST

GIST is generally recognized as a glycolysis-dependent tumor, which can be evaluated for the metabolic activity in FDG-PET scan. A few studies have investigated the energy metabolism in GIST, especially their regulation upon imatinib treatment [167]. Vitiello et al. found that imatinib suppresses glycolysis and induces OXPHOS-associated gene expressions. This study revealed the evidence of metabolic reprogramming toward OXPHOS upon imatinib stress and demonstrated the rationale of using a combination of OXPHOS inhibitor and imatinib for GIST therapy [168]. Our study further characterized the bioenergetic profile in imatinib-resistant GIST cells. We found that imatinib-resistant GIST cells can harbor high metabolic status (upregulated high glycolysis and OXPHOS status) and low OXPHOS status, suggesting the heterogeneity of metabolic reprogramming in GIST cells surviving imatinib cytotoxicity [169].

1.4.3 Energy metabolism in colorectal cancer (CRC)

Several studies revealed upregulated OXPHOS in (CRC). A bioenergetic profiling study revealed a similar glycolytic activity and an upregulation of OXPHOS in CRC tissues compared to the surrounding healthy colon tissue [170]. The mitochondrial pyruvate carrier (MPC), a transporter for the mitochondrial uptake of pyruvate, was found to be upregulated in CRC and thereby abrogated the Warburg effect and facilitated the oxidative metabolism [171]. Chemoresistance to 5-fluorouracil (5-FU) in colon cancer cells exhibited upregulated OXPHOS and mitochondrial biomass and higher oxygen consumption rates via the histone deacetylase sirtuin-1 (SIRT1) and the downstream transcriptional coactivator PGC1α pathway [172]. The OXPHOS inhibitor metformin in combination with 5-FU showed a high sensitivity to the resistant colon cancer cells with stem-like features [173]. Furthermore, the different subgroups of CRC by molecular types demonstrated distinct types of energy metabolism. In

the *BRAF* or *KRAS* mutated tumors, metabolic reprogramming shifts from OXPHOS to a more glycolytic type [174].

2 AIMS OF THE THESIS

The overall aim of this thesis work was to understand the roles of miRNA and energy metabolism in treatment response in gastrointestinal tumors. More specifically, we aimed to:

Paper I: Identify the downstream target of *miR-125a-5p*-PTPN18 pathway in regulating imatinib response in GIST.

Paper II: Characterize the bioenergetic profile of imatinib-resistant GIST.

Paper III: Determine whether miRNAs are involved in imatinib-induced OXPHOS in GIST.

Paper IV: Evaluate the association of metformin use with mortality in colorectal cancer patients.

3 MATERIALS AND METHODS

3.1 CELL LINES

Five established human GIST cell lines were included in this thesis. Two are imatinib-sensitive cell lines. GIST 882 contains a homozygous missense mutation in *KIT* exon 13 (K642E) **(Papers I-III)**. GIST T1 has a heterozygous 57-bp deletion in *KIT* exon 11. Three imatinib-resistant cell lines GIST 882R **(Papers I and II)** and GIST T1R **(Paper II)**, which were developed from parental GIST 882 and GIST T1 cells, and GIST 48, harboring a primary homozygous *KIT* exon 11 missense mutation (V560D) and a secondary heterozygous *KIT* exon 17 (D820A) mutation **(Paper II)**. GIST 882 and GIST 48 were kindly provided by Dr. Jonathan Fletcher (Brigham and Women's Hospital, Boston, MA, USA). GIST T1 was purchased from Cosmo Bio Co. Ltd. (Tokyo, Japan). The authenticity of the cell lines was confirmed by short tandem repeat (STR) genotyping, as described in **Paper II**.

3.2 TUMOR SAMPLES

In total, 64 snap-frozen tumors derived from 57 GIST patients were included in this thesis. Among these tumors, 30 had neoadjuvant imatinib treatment, referring to the imatinib-treated group **(Papers I-II)**, and 34 tumors were not treated with imatinib treatment prior to surgery **(Papers II and III)**. Among the imatinib-treated group, 14 tumors were imatinib resistant, whereas 16 tumors were imatinib sensitive. The clinical, histopathological and follow-up details of the cases are detailed in **Papers I-II** and in [138].

3.3 RNA DETECTION AND QUANTIFICATION METHODS

Total RNA, including miRNA, was extracted by using miRVana miRNA isolation kit (Thermo Fisher Scientific, Waltham, MA). The RNA concentrations were determined by a NanoDrop spectrophotometer (NanoDrop technologies, Wilmington, DE).

3.3.1 Reverse transcription-quantitative polymerase chain reaction

Total RNA was reverse transcribed to first-strand cDNA using the TaqMan Multiscribe Reverse Transcription system. Two types of reverse transcription quantitative polymerase chain reaction (RT-qPCR), SYBR green and TaqMan assays, are reliable methods for RNA quantification. We have chosen the TaqMan assay (Applied Biosystems), which uses fluorophore (FAM)-labeled TaqMan probes to quantify the gene expression. The amount of FAM fluorescence signals during PCR amplification was measured as a function of PCR cycle number using an ABI 7900 Real-time PCR System (Applied Biosystems). This method was performed to detect *miR-125a-5p* and *RNU6B* in **Paper I**, and *miR-320a*, *miR320b*, *miR-193a-3p*, *miR-483-3p* and *RNU48* in **Paper III.**

3.3.2 miRNA microarray

Global miRNA expression profiles in **Paper III** were determined using the Agilent human miRNA microarray platform (Agilent Technologies, Santa Clara, CA, USA). This platform provides high sensitivity and specificity using the unique design of the probes matching 903 human miRNAs (miRBase release 14).

3.4 WESTERN BLOT ANALYSIS

Western blot analysis is a widely used method for quantification and detection of specific proteins. Cells or tissue specimens were lysed in Nonidet-P40 (NP40) lysis buffer supplemented with 1 mM phenylmethysulfonyl fluoride and protease inhibitors. Lysates were loaded and separated using SDS-PAGE system with 10% or 4-12% Bis-Tris gels and then transferred to nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween-20, and then followed by incubating with primary antibodies for proteins of interest overnight at 4 °C. After washing, the membrane was incubated with host-matched secondary antibody at room temperature, and visualized by chemiluminescent or fluorescent detection. This method was used to analyze PTPN18, phosphorylated FAK (pFAK), FAK, and pSTAT3 (**Paper I**), AKT, phosphorylated AKT at Ser473, PGC1α, mitochondrial transcription factor A (TFAM), LDHA, LDHB, total OXPHOS, HK1, HK2, NRF1, NRF2, poly (ADP-ribose) polymerase (PARP) (**Paper II**) and SDHB (**Paper III**).

3.5 TRANSFECTION

Cell transfection is a method wherein foreign genetic molecules, such as synthesized or recombinant nucleic acids, are delivered into cells. The transfection methods include electrical transfection techniques such as electroporation, biochemical methods such as calcium phosphate co-precipitation, lipid-based reagents, and biologic methods such as virus-based vectors. Electroporation was used in this thesis for delivery of miRNA mimics/inhibitors and short hairpin RNA. We used Amaxa nucleofection system (Lonza), an electroporation method combined with a cell-specific transfection medium that facilitates nuclear delivery, especially suitable for difficult-to-transfect cells.

3.5.1 miRNA mimics and inhibitors

MiRNA mimics are chemically synthesized double-stranded RNA molecules that mimic endogenous mature miRNA. Anti-miR miRNA inhibitors are single-stranded nucleic acids designed to specifically bind to and inhibit endogenous miRNA. In **Paper I**, miRNA

mimics (pre-miR-125a-5p or pre-miR negative control) was transfected into GIST 882 cells. In **paper III**, *miR-483-3p* mimics and inhibitors were transfected into GIST 882 and GIST T1 cells.

3.5.2 Short hairpin RNA

We used short hairpin RNA (shRNA) for silencing gene expression. shRNA targeting PTPN18 (target sequence: 5'-GAGGGACACAGCGACTACA-3') was cloned into pcDNA3-U6M2 vector [138]. Generally, a loop transcript consisting of sense and antisense sequences was generated upon transcription. The loop structure was recognized by the Dicer enzyme to produce mature active siRNA. In **Paper I**, shRNA against PTPN18 or vector control (shControl) was transfected into GIST 882 cells.

3.5.3 Small interfering RNA

Unlike shRNA as vector-based RNA interference, small interfering RNA (siRNA) is chemically synthesized double-stranded RNA. Although they are commonly used for gene knockdown, they are different in several ways. SiRNAs are oligonucleotides and thereby directly loaded into the RNA-induced silencing complex (RISC) in the cytoplasm, while shRNAs are transcribed in the nucleus and processed by Dicer before loading into RISC. Furthermore, siRNAs generally confer short-term effect, while shRNAs have longer-term effect and can be used for stable transfection.

3.6 FUNCTIONAL ASSAYS

In this thesis, several assays were used for studies of cell viability, proliferation, and apoptosis.

3.6.1 Cell viability

Cell viability can be measured using the metabolic activity in viable cells that reduces tetrazolium salts such as WST-1 ((4-(3-(4-iodophenyl)-2-(4nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate) into a colored soluble formazan dye. The colored dye allows quantification by spectrophotometer, which is proportional to the activity of the mitochondrial dehydrogenase system in viable cells. Comparing to other tetrazolium salts such as MTT and MTS, the WST-1 assay is less toxic, more sensitive and stable. The WST-1 assay was used in **Paper I** and **Paper II**.

3.6.2 Cell proliferation

Several assays are commonly used for cell proliferation analysis. First, DNA synthesis methods measure a labeled DNA analog or precursor such as 5-bromo-2'-deoxyuridine (BrdU)

or ethynyl-deoxyuridine (EdU), a pyrimidine analog or [^{3}H]-thymidine incorporating into newly synthesized DNA during the S phase of the cell cycle. Second, IHC analysis for markers of proliferation can be applied such as Ki-67, proliferating cell nuclear antigen (PCNA), or minichromosome maintenance complex component (MCM). Moreover, real-time live cell imaging can also provide a measurement of the cell proliferation. For example, time-lapse phase-contrast imaging using Incucyte Live Cell Imaging (Essen Biosciences) can monitor the cell growth in a real-time manner. In this thesis, we used Incucyte imaging system to monitor the cell proliferation in **Paper II**.

3.6.3 Apoptosis

Apoptosis is a form of programmed cell death mediated by caspase activation upon external or internal stress. Several methods can assess the apoptotic cells such as microscopic morphology examination, terminal-deoxynucleotidyl transferase mediated nick end labelling (TUNEL) staining assay, Annexin V fluorescence staining, caspase-3 activity ELISA activity, JC-1 staining to detect mitochondrial membrane potential (MMP), and Western blot analysis for apoptotic markers. In this thesis, we used Annexin V/ propidium iodide (AV/PI) fluorescence staining (**Paper II**) and Western blotting for cleaved PARP1 (**Papers I and II**) to assess apoptosis. The AV/PI staining can be used to distinguish early stages of apoptosis (AV positive and PI negative) and late apoptosis/necrosis (both AV and PI positive). The externalization of phosphatidylserine on the cell membrane prior to the loss of cell membrane integrity during apoptosis can be recognized by AV and PI, respectively. Therefore, in early stage of apoptosis cells are AV positive and PI negative, while in late apoptosis cells are both positive for AV and PI.

PARP-1, a key enzyme cleaves NAD+ into nicotinamide and ADP-ribose, is crucial for synthesis of protein-bound ADPR polymers upon single-strand DNA break. PARP-1 is a substrate of caspase-3. Therefore, the cleavage of PARP-1 is an indicator of apoptosis, which can be detected by Western blotting.

3.7 METABOLIC ASSAYS

In this thesis, we measured the glycolysis, OXPHOS, mitochondrial mass, and reactive oxygen species (ROS) using several different assays.

3.7.1 Mitochondrial bioenergetics

The mitochondrial bioenergetics profile was performed by sequential injections of an ATP synthase inhibitor (oligomycin), a mitochondrial uncoupling agent (carbonyl cyanide-4-(trifluoromethoxy)phenyl-hydrazone (FCCP), and respiratory chain inhibitors (antimycin A and rotenone). These serial additions can assess five mitochondrial oxygen consumption rates, including basal respiration, proton leak, ATP production, maximal respiration capacity, and spare respiratory capacity by using Seahorse XF96 analyzer. In **Paper II**, we used these parameters to assess the OXPHOS activity.

3.7.2 Glycolysis activity

Several assays can be used to monitor glycolysis. Extracellular glucose or lactate can be measured using spectrophotometry, which is simple and standard. Using extracellular bioanalyzer is also simple. Extracellular acidification rate (ECAR) measurement using seahorse XF analyzer can provide not only ECAR and simultaneous oxygen consumption rate (OCR) information. As lactate, the end product of glycolysis, is the source of acidification, we can measure ECAR to assess the glycolysis activity. We performed glycolysis stress test using Seahorse XF96 analyzer to measure the glycolysis activity in **Paper II**.

3.7.3 Mitochondrial mass measurement

Relative mitochondrial mass can be measured by the ratio of mitochondrial and nuclear DNA (mtDNA/nDNA), immunoblotting of key mitochondrial proteins, measuring mitochondrial protein content or weight after mitochondrial isolation, and MitoTracker Green. In this thesis, we measured mitochondrial mass of GIST cells by using MitoTracker Green, which is a mitochondrial mass-dependent fluorescence regardless of mitochondrial membrane potential, in **Paper II**.

3.7.4 Intracellular reactive oxygen species

Several assays can be used for measuring intracellular ROS such as Mito-SOX and dichlorodihydrofluorescein diacetate, which detects superoxide and hydrogen peroxide, respectively. In this thesis, we used dichlorodihydrofluorescein diacetate to assess whole-cell ROS level in **Paper II.**

3.7.5 Glucose uptake

Two types of glucose analogs are commonly used for measuring glucose uptake. One is radioactive glucose analogs, such as 2-deoxy-d-[1,2-^{3}H]-glucose (2-DG), or 2-deoxy-2-(^{18}F)-fluoro-d-glucose (^{18}FDG). The other is fluorescent glucose analogs, such as 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG). In this thesis, we used 2-NBDG assay for glucose uptake measurement in **Paper II**.

3.8 IMMUNOFLUORESCENCE

Immunofluorescence can provide information about expression intensity and subcellular localization information of target proteins. We used immunofluorescence imaging for analyzing the expression and subcellular localization of PGC1α in **Paper II**.

3.9 INHIBITION OF FAK PHOSPHORYLATION

The phosphorylation sites of FAK include Tyr397, Tyr576/577, Tyr861, and Tyr925. FAK phosphorylated at Tyr397 is a major transducer to engage integrin-mediated survival signals. FAK Inhibitor 14 (Sigma) is a cell-permeable selective FAK inhibitor that specifically blocks phosphorylation of Tyr397. Therefore, we used this chemical for inhibition of FAK phosphorylation in **Paper I**.

3.10 INHIBITION OF GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION

Several classes of glycolysis inhibitors target different steps of glycolysis. For example, glucose analog 2-deoxyglucose (2-DG), 3-Bromopyruvate (3-BP) and lonidamine are HK2 inhibitors, and 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) is a fructose 2,6-bisphosphate (F-2,6-BP) inhibitor such as TEPP-46 and DASA-58 are small molecule inhibitors of pyruvate kinase isozyme 2 (PKM2). Lactate dehydrogenase (LDH) inhibitors include FX11, gossypol, and oxamate. In this thesis, we used 3-BP and gossypol for glycolysis inhibition in **Paper II**.

There are several inhibitors target different OXPHOS complexes such as metformin and rotenone for Complex I, antimycin A for Complex III, sodium azide for Complex IV, or oligomycin for Complex V. We used oligomycin and antimycin A as the OXPHOS inhibitors in **Paper II**.

3.11 STATISTICAL ANALYSIS

All statistical tests in this thesis were performed using GraphPad Prism 8.0 or Microsoft Office Excel 2010. For miRNA microarray analysis, Significance Analysis of Microarray (SAM) (http://statweb.stanford.edu/~tibs/SAM/) was used to determine the most significant differentially expressed miRNAs between the imatinib-treated and non-treated samples in **Paper III**. Statistical significance levels were assessed by using the false discovery rate (FDR).

Independent Student's *t*-test, Mann-Whitney *U*-test, or one-way ANOVA (more than two groups) was performed to compare the expression of miRNA and protein in different patient groups or cell lines (**Papers I-III**). Paired *t*-test was used to compare two different conditions between the experimental groups (**Papers I and III**). A two-way ANOVA was applied for more than two groups of two variables, followed by a post-hoc test (Dunnett's). In **Paper IV**, baseline characteristics are shown as numbers and percentages for categorical variables and as medians and quantiles for continuous variables. Time-dependent Cox regression models were applied to obtain hazard ratios and 95% confidence intervals. Stabilized inverse probability of treatment weighting (IPTW) using propensity scores was used to balance baseline covariates between metformin users and nonusers. All p values are based on two-tailed tests. Significance is indicated as *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$.

4 RESULTS AND DISCUSSION

4.1 *MIR-125A-5P* REGULATION OF FAK PHOSPHORYLATION IN IMATINIB RESISTANCE OF GASTROINTESTINAL STROMAL TUMORS (PAPER I)

In our previous study, we identified *miR-125a-5p* as a regulator of imatinib response via targeting PTPN18. However, the downstream mechanism involved in altered imatinib response was not clear. FAK has been shown to play a significant role in promoting GIST progression and is associated with poor prognosis. We therefore investigated whether phosphorylation of FAK is the downstream target of PTPN18 and *miR-125a-5p*.

4.1.1 Regulation of FAK phosphorylation by *miR-125a-5p* and PTPN18

We found that silencing of PTPN18 by shPTPN18 upregulates the expression of pFAK but not phosphorylated STAT3. Similarly, siPTPN18 transfection using two different siRNAs also upregulated pFAK but not the total FAK level, suggesting that pFAK is the specific substrate of PTPN18. Furthermore, overexpression of *miR-125a-5p* led to increased expression of pFAK. These findings suggest that *miR-125a-5p* may upregulate pFAK via silencing of PTPN18.

4.1.2 FAK phosphorylation and imatinib resistance in GIST

In imatinib-resistant cell lines, we found that *miR-125a-5p* expressions were upregulated compared to their parental imatinib-sensitive cell line. Higher *miR-125a-5p* expression in the imatinib-resistant cell lines was concordant with lower PTPN18 and higher pFAK expressions. The FAK levels were similar between the imatinib-resistant and -sensitive cell lines. To test the role of pFAK, we used FAK inhibitor 14, an inhibitor of FAK that specifically blocks phosphorylation at tyrosine residue of codon 397. Inhibition of pFAK led to increased apoptosis and reduced cell viability in imatinib-resistant GIST cell lines, as evaluated by detection of cleaved PARP and WST-1 assay, respectively. Furthermore, FAK inhibitor 14 could re-sensitize resistant cell lines to imatinib and reversed the imatinib resistance effect contributed by *miR-125a-5p* overexpression. These results suggest that FAK phosphorylation is critical in maintaining growth in imatinib-resistant GIST cells.

4.1.3 Association of pFAK and FAK levels with clinical outcomes

We further examined the association between FAK or pFAK and clinical outcomes. We found that upregulated FAK expression is associated with metastasis in GISTs, indicating its role in tumor progression. However, we did not find a significant association of pFAK with tumor progression, which is consistent with the study by Kamo et al. [175], suggesting that FAK may contribute to tumor progression in a kinase-independent manner.

In summary, we demonstrated the link between *miR-125a-5p*-PTPN18 axis and pFAK, which involves in regulating imatinib response in GIST cells. The total FAK level is associated with GIST tumor progression.

4.2 METABOLIC HETEROGENEITY IN IMATINIB RESISTANT GIST (PAPER II)

A previous study demonstrated that imatinib sensitive GIST cells undergo metabolic reprogramming to OXPHOS upon imatinib treatment, and inhibition of OXPHOS in combination with imatinib can enhance imatinib cytotoxicity. However, the bioenergetic profile of imatinib-resistant GIST cells is not well understood. Therefore we further profiled the energy metabolism of imatinib-resistant GIST cells and investigated the effect of metabolic inhibitors on imatinib-resistant GIST cell lines.

4.2.1 Effect of imatinib treatment on OXPHOS and mitochondrial biogenesis

First, we tested the effect of imatinib on metabolic reprogramming in imatinib-sensitive and -resistant GIST cell lines. OXPHOS proteins were increased in both imatinib-sensitive cell lines (GIST T1 and 882) upon imatinib treatment, which are consistent to the study by Vitiello et al. [168]. However, no significant alteration of OXPHOS proteins were found in the imatinib-resistant GIST T1 and 882 cells upon imatinib treatment, suggesting that metabolic adaptation to imatinib did not occur in imatinib-resistant GIST. Since the mitochondrial biogenesis is closely correlated to OXPHOS activity, we further examined the mitochondrial mass and two key regulator proteins, mitochondrial transcription factor A (TFAM) and PGC1α using immunoblot analysis, immunofluorescence, and flow cytometry. We found that the expressions of TFAM and PGC1α were downregulated in both imatinib-sensitive cell lines upon imatinib treatment. However, the alterations were not consistent in imatinib-resistant GIST cell lines. In imatinib-resistant GIST T1 cells, both TFAM and PGC1α expressions were downregulated, while no significant changes were found in GIST 882R cells. These findings may indicate different mitochondrial response to imatinib in resistant GIST cells. In clinical samples, we found significant downregulation of TFAM in both sensitive and resistant imatinib-treated GIST tumors.

4.2.2 Metabolic heterogeneity in GIST cell lines and tumor samples

We further characterized the energy metabolism of imatinib-resistant GIST cells, including two acquired resistant cell lines (GIST 882R and GIST T1R). We found that GIST 882R and GIST 48 cells were more glycolytic than the parental GIST 882 cells. However, GIST T1R cells had similar basal glycolysis and less glycolytic activity than GIST T1 cells. Furthermore, GIST 882R cells also had more OXPHOS activities in terms of basal respiration and maximal respiratory

capacity compared with GIST 882 cells. On the other hand, GIST T1R cells had less OXPHOS activity than GIST T1 cells. These results suggest heterogeneity of metabolic reprogramming in imatinib-resistant GIST cells. GIST 882R cells were highly metabolically active (glycolysis-high, OXPHOS-high) compared with GIST 882 cells, while reduced OXPHOS activity was found in GIST T1R cells. In concordance with the observation in cell lines, microarray expression profiles of 15 imatinib-resistant GIST samples revealed two distinct clusters based on the expression of glycolysis and OXPHOS genes. One cluster displayed both higher expressions of glycolysis and OXPHOS genes, suggesting a highly metabolically active phenotype. The other cluster showed a relatively low OXPHOS status. Together, our results suggest metabolic heterogeneity in imatinib-resistant GISTs.

4.2.3 Inhibition of glycolysis and oxidative phosphorylation on cell viability

Given that GIST 882R cells were more glycolytic than GIST 882 cells, we further tested whether they are more susceptible to glycolysis inhibitors. We found that 100 μM 3-BP, a hexokinase 2 inhibitor, induced significant apoptosis and inhibited the growth in GIST 882R cells and GIST 48 cells, but not in GIST 882 cells. Similarly, 10 μM gossypol, a LDHA inhibitor, reduced the proliferation of GIST 882R cells, but not GIST 882 cells. These results demonstrate that imatinib-resistant GIST cells with more glycolysis activity are vulnerable to inhibition of glycolysis. On the other hand, GIST T1R and GIST T1 cells did not show different cell growth upon 3-BP treatment. However, GIST T1R cells were more resistant to both antimycin A and oligomycin than GIST T1 cells.

In summary, we found that resistant cells can manifest a highly metabolic active phenotype or a relatively low OXPHOS state, which results in differences for metabolic vulnerability in imatinib-resistant GIST cells.

4.3 IMATINIB INDUCED DOWNREGULATION OF *MIR-483-3P* AND UPREGULATION OF SDH IN GIST (PAPER III)

In **Paper II**, we found upregulation of OXPHOS in imatinib-sensitive GIST cells upon imatinib treatment. This led us to further investigate the potential role of miRNAs in regulating OXPHOS. First we compared global miRNA expression profiles between imatinib-treated and untreated GIST samples.

4.3.1 Deregulation of miRNA induced by imatinib

We characterized global miRNA expression profiles of 19 imatinib-treated and 15 untreated tumors using a microarray-based approach. Several miRNAs were deregulated in imatinib-treated tumors including overexpression of *miR-320a*, *miR-320b* and *miR-193a-3p* and reduced expression of *miR-483-3p* and *miR-149*. We confirmed significantly upregulation of *miR-320b* and downregulation of *miR-483-3p* in imatinib-treated tumors by RT-qPCR in an extended cohort. In concordance with the results of microarray and RT-qPCR, we found downregulation of *miR-483-3p* in GIST cells upon imatinib treatment. These results suggest that imatinib induces deregulation of selected miRNAs, which may participate in biological processes upon imatinib treatment, such as metabolic reprogramming to OXPHOS.

4.3.2 *miR-483-3p* regulates Complex II expression

Overexpression of *miR-483-3p* suppressed, while inhibition of *miR-483-3p* upregulated Complex II expression, as indicated by increased SDHB expression. Of note, Complex III and V were not altered by modulation of *miR-483-3p*. We found an inverse relationship between *miR-483-3p* and SDHB protein expression among GIST tumor samples. These results suggest that SDH may be a potential target of *miR-483-3p*. The mechanistic regulation between *miR-483-3p* and SDH warrants further investigation.

4.4 METFORMIN ASSOCIATED WITH IMPROVING SURVIVAL IN CRC (PAPER IV)

Preclinical evidence indicates the anti-tumor effect of metformin in CRC. However, previous epidemiologic results remained controversial. Here we presented a large-scale nationwide cohort study to investigate the association of post-diagnostic metformin use with the mortality.

4.4.1 Characteristics of metformin users versus nonusers

Between 2004 and 2014, 16,676 diabetic patients were diagnosed with CRC, including metformin nonusers (n = 5,238) and users (n = 11,438). We found that metformin users were younger, had lower score of comorbidities, and less advanced diseases. These results indicated that prescription of anti-diabetic medications may differ based on patients' baseline characteristics. To reduce the baseline imbalance, we used the inverse probability of treatment weighting method in this study.

4.4.2 Metformin use and improving survival in patients with CRC

After adjustment for confounders and baseline characteristics using inverse probability of treatment weighting, metformin users had a 58% reduced risk of all-cause mortality than the nonusers. They also had a lower CRC-specific mortality with the hazard ratio of 0.41 (95%CI, 0.39-0.44). The results were not altered across the subgroup analyses, indicating the consistent findings regardless of subpopulations. Overall, our conclusion suggests that metformin can be considered as an adjunct to the standard care in patients with CRC.

5 CONCLUSIONS

In this thesis we provide insights into the role of miRNA and energy metabolism in regulating drug response in gastrointestinal malignancy. The main findings are as follows:

- The *miR-125a-5p*-PTPN18 axis regulates imatinib response in GIST cells via targeting pFAK (**Paper I**).
- Inhibition of pFAK can re-sensitize the imatinib resistance induced by overexpression of *miR-125a-5p* (**Paper I**).
- Imatinib-resistant GIST cells manifest two distinct types of energy metabolism, one shows a highly metabolically active phenotype and the other has low expression of OXPHOS (**Paper II**).
- Imatinib-resistant GIST cells with higher glycolysis activity are more sensitive to glycolysis inhibitors compared with their parental GIST cells. Imatinib-resistant GIST cells with lower OXPHOS activity are more resistant to OXPHOS inhibitors than their parental cells (**Paper II**).
- OXPHOS is upregaulted upon imatinib treatment in imatinib-sensitive GIST cells (**Paper II**). Selected miRNAs are deregulated, such as downregulated *miR-483-3p*, between imatinib-treated and untreated GIST cells (**Paper III**).
- *miR-483-3p* regulates the expression of SDH, which is the Complex II of the mitochondrial respiratory chain. Moreover, the expression of *miR-483-3p* was inversely correlated with SDH in GIST tumors (**Paper III**).
- Postdiagnostic metformin use, an inhibitor of OXPHOS Complex I, is associated with better prognosis in diabetic patients with colorectal cancer (**Paper IV**).
- Targeting energy metabolism and miRNA therapeutics can be potential to improve targeted drug resistance and survival in gastrointestinal malignancy.

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7 REFERENCES

[1] Song JH, Meltzer SJ. MicroRNAs in pathogenesis, diagnosis, and treatment of gastroesophageal cancers. Gastroenterology 2012;143:35-47 e2.

[2] Soreide K, Sandvik OM, Soreide JA, Giljaca V, Jureckova A, Bulusu VR. Global epidemiology of gastrointestinal stromal tumours (GIST): A systematic review of population-based cohort studies. Cancer Epidemiol 2016;40:39-46.

[3] Wang ZH, Liang XB, Wang Y, Ma GL, Qu YQ, Tian XW. [Epidemiology survey of gastrointestinal stromal tumor in Shanxi Province in 2011]. Zhonghua Yi Xue Za Zhi 2013;93:2541-4.

[4] Brabec P, Sufliarsky J, Linke Z, Plank L, Mrhalova M, Pavlik T, et al. A whole population study of gastrointestinal stromal tumors in the Czech Republic and Slovakia. Neoplasma 2009;56:459-64.

[5] Cho MY, Sohn JH, Kim JM, Kim KM, Park YS, Kim WH, et al. Current trends in the epidemiological and pathological characteristics of gastrointestinal stromal tumors in Korea, 2003-2004. J Korean Med Sci 2010;25:853-62.

[6] Chiang NJ, Chen LT, Tsai CR, Chang JS. The epidemiology of gastrointestinal stromal tumors in Taiwan, 1998-2008: a nation-wide cancer registry-based study. BMC Cancer 2014;14:102.

[7] Chan KH, Chan CW, Chow WH, Kwan WK, Kong CK, Mak KF, et al. Gastrointestinal stromal tumors in a cohort of Chinese patients in Hong Kong. World J Gastroenterol 2006;12:2223-8.

[8] Tran T, Davila JA, El-Serag HB. The epidemiology of malignant gastrointestinal stromal tumors: an analysis of 1,458 cases from 1992 to 2000. Am J Gastroenterol 2005;100:162-8.

[9] Nilsson B, Bumming P, Meis-Kindblom JM, Oden A, Dortok A, Gustavsson B, et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western Sweden. Cancer 2005;103:821-9.

[10] Goettsch WG, Bos SD, Breekveldt-Postma N, Casparie M, Herings RM, Hogendoorn PC. Incidence of gastrointestinal stromal tumours is underestimated: results of a nation-wide study. Eur J Cancer 2005;41:2868-72.

[11] Mazur MT, Clark HB. Gastric stromal tumors. Reappraisal of histogenesis. Am J Surg Pathol 1983;7:507-19.

[12] Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998;152:1259-69.

[13] Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279:577-80.

[14] Tryggvason G, Gislason HG, Magnusson MK, Jonasson JG. Gastrointestinal stromal tumors in Iceland, 1990-2003: the icelandic GIST study, a population-based incidence and pathologic risk stratification study. Int J Cancer 2005;117:289-93.

[15] Miettinen M, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. Virchows Arch 2001;438:1-12.

[16] Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, Nakajima T. PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. Pathol Int 2005;55:106-12.

[17] West RB, Corless CL, Chen X, Rubin BP, Subramanian S, Montgomery K, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. Am J Pathol 2004;165:107-13.

[18] Blay JY, Bonvalot S, Casali P, Choi H, Debiec-Richter M, Dei Tos AP, et al. Consensus meeting for the management of gastrointestinal stromal tumors. Report of the GIST Consensus Conference of 20-21 March 2004, under the auspices of ESMO. Ann Oncol 2005;16:566-78.

[19] Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer 2011;11:865-78.

[20] Rossi S, Gasparotto D, Toffolatti L, Pastrello C, Gallina G, Marzotto A, et al. Molecular and clinicopathologic characterization of gastrointestinal stromal tumors (GISTs) of small size. Am J Surg Pathol 2010;34:1480-91.

[21] Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004;22:3813-25.

[22] Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 2005;23:5357-64.

[23] Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 2003;21:4342-9.

[24] Wada R, Arai H, Kure S, Peng WX, Naito Z. "Wild type" GIST: Clinicopathological features and clinical practice. Pathol Int 2016;66:431-7.

[25] Miettinen M, Wang ZF, Sarlomo-Rikala M, Osuch C, Rutkowski P, Lasota J. Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. Am J Surg Pathol 2011;35:1712-21.

[26] Andersson J, Sihto H, Meis-Kindblom JM, Joensuu H, Nupponen N, Kindblom LG. NF1-associated gastrointestinal stromal tumors have unique clinical, phenotypic, and genotypic characteristics. Am J Surg Pathol 2005;29:1170-6.

[27] Ricci R. Syndromic gastrointestinal stromal tumors. Hered Cancer Clin Pract 2016;14:15.

[28] Haller F, Moskalev EA, Faucz FR, Barthelmess S, Wiemann S, Bieg M, et al. Aberrant DNA hypermethylation of SDHC: a novel mechanism of tumor development in Carney triad. Endocr Relat Cancer 2014;21:567-77.

[29] Sandvik OM, Soreide K, Kvaloy JT, Gudlaugsson E, Soreide JA. Epidemiology of gastrointestinal stromal tumours: single-institution experience and clinical presentation over three decades. Cancer Epidemiol 2011;35:515-20.

[30] Miettinen M, Monihan JM, Sarlomo-Rikala M, Kovatich AJ, Carr NJ, Emory TS, et al. Gastrointestinal stromal tumors/smooth muscle tumors (GISTs) primary in the omentum and

mesentery: clinicopathologic and immunohistochemical study of 26 cases. Am J Surg Pathol 1999;23:1109-18.

[31] Emile JF, Brahimi S, Coindre JM, Bringuier PP, Monges G, Samb P, et al. Frequencies of KIT and PDGFRA mutations in the MolecGIST prospective population-based study differ from those of advanced GISTs. Med Oncol 2012;29:1765-72.

[32] Gold JS, Gonen M, Gutierrez A, Broto JM, Garcia-del-Muro X, Smyrk TC, et al. Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localised primary gastrointestinal stromal tumour: a retrospective analysis. Lancet Oncol 2009;10:1045-52.

[33] Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. Lancet 2007;369:1731-41.

[34] Crosby JA, Catton CN, Davis A, Couture J, O'Sullivan B, Kandel R, et al. Malignant gastrointestinal stromal tumors of the small intestine: a review of 50 cases from a prospective database. Ann Surg Oncol 2001;8:50-9.

[35] Hassan I, You YN, Shyyan R, Dozois EJ, Smyrk TC, Okuno SH, et al. Surgically managed gastrointestinal stromal tumors: a comparative and prognostic analysis. Ann Surg Oncol 2008;15:52-9.

[36] Joensuu H, Hohenberger P, Corless CL. Gastrointestinal stromal tumour. Lancet 2013;382:973-83.

[37] Group ESESNW. Gastrointestinal stromal tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014;25 Suppl 3:iii21-6.

[38] Wu PC, Langerman A, Ryan CW, Hart J, Swiger S, Posner MC. Surgical treatment of gastrointestinal stromal tumors in the imatinib (STI-571) era. Surgery 2003;134:656-65; discussion 65-6.

[39] DeMatteo RP, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg 2000;231:51-8.

[40] Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay JY, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 2004;364:1127-34.

[41] Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol 2008;26:626-32.

[42] Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 2006;368:1329-38.

[43] Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, et al. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013;381:295-302.

[44] Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, Koryotowski B, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 2005;11:4182-90.

[45] Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 2006;24:4764-74.

[46] Wardelmann E, Merkelbach-Bruse S, Pauls K, Thomas N, Schildhaus HU, Heinicke T, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 2006;12:1743-9.

[47] Nishida T, Kanda T, Nishitani A, Takahashi T, Nakajima K, Ishikawa T, et al. Secondary mutations in the kinase domain of the KIT gene are predominant in imatinib-resistant gastrointestinal stromal tumor. Cancer Sci 2008;99:799-804.

[48] Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N, et al. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 2005;128:270-9.

[49] Takahashi T, Serada S, Ako M, Fujimoto M, Miyazaki Y, Nakatsuka R, et al. New findings of kinase switching in gastrointestinal stromal tumor under imatinib using phosphoproteomic analysis. Int J Cancer 2013;133:2737-43.

[50] Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, et al. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. Oncogene 2007;26:3909-19.

[51] Rossi F, Yozgat Y, de Stanchina E, Veach D, Clarkson B, Manova K, et al. Imatinib upregulates compensatory integrin signaling in a mouse model of gastrointestinal stromal tumor and is more effective when combined with dasatinib. Mol Cancer Res 2010;8:1271-83.

[52] Cohen NA, Zeng S, Seifert AM, Kim TS, Sorenson EC, Greer JB, et al. Pharmacological Inhibition of KIT Activates MET Signaling in Gastrointestinal Stromal Tumors. Cancer Res 2015;75:2061-70.

[53] Javidi-Sharifi N, Traer E, Martinez J, Gupta A, Taguchi T, Dunlap J, et al. Crosstalk between KIT and FGFR3 Promotes Gastrointestinal Stromal Tumor Cell Growth and Drug Resistance. Cancer Res 2015;75:880-91.

[54] Antonescu CR, Romeo S, Zhang L, Nafa K, Hornick JL, Nielsen GP, et al. Dedifferentiation in gastrointestinal stromal tumor to an anaplastic KIT-negative phenotype: a diagnostic pitfall: morphologic and molecular characterization of 8 cases occurring either de novo or after imatinib therapy. Am J Surg Pathol 2013;37:385-92.

[55] Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 2003;10:159-65.

[56] Smith BD, Kaufman MD, Lu WP, Gupta A, Leary CB, Wise SC, et al. Ripretinib (DCC-2618) Is a Switch Control Kinase Inhibitor of a Broad Spectrum of Oncogenic and Drug-Resistant KIT and PDGFRA Variants. Cancer Cell 2019;35:738-51 e9.

[57] Blay JY, Serrano C, Heinrich MC, Zalcberg J, Bauer S, Gelderblom H, et al. Ripretinib in patients with advanced gastrointestinal stromal tumours (INVICTUS): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol 2020;21:923-34.

[58] Heinrich M. Clinical activity of avapritinib in ≥ fourth-line (4L+) and PDGFRA Exon 18 gastrointestinal stromal tumors (GIST). Journal of Clinical Oncology 37, no 15_suppl (May 20, 2019) 11022-11022.

[59] Schoffski S. Activity and safety of cabozantinib in patients with gastrointestinal stromal tumor after failure of imatinib and sunitinib: EORTC phase II trial 1317 CaboGIST. . Journal of Clinical Oncology 37, no 15_suppl (May 20, 2019) 11006-11006.

[60] miRBase: the microRNA database.

[61] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer 2015;15:321-33.

[62] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002;99:15524-9.

[63] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-8.

[64] Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25:2537-45.

[65] Schepeler T, Reinert JT, Ostenfeld MS, Christensen LL, Silahtaroglu AN, Dyrskjot L, et al. Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer Res 2008;68:6416-24.

[66] Gottardo F, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P, et al. Micro-RNA profiling in kidney and bladder cancers. Urol Oncol 2007;25:387-92.

[67] Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. Oncogene 2006;25:6202-10.

[68] Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005;65:9628-32.

[69] O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839-43.

[70] Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, et al. An E2F/miR-20a autoregulatory feedback loop. J Biol Chem 2007;282:2135-43.

[71] Wang B, Hsu SH, Wang X, Kutay H, Bid HK, Yu J, et al. Reciprocal regulation of microRNA-122 and c-Myc in hepatocellular cancer: role of E2F1 and transcription factor dimerization partner 2. Hepatology 2014;59:555-66.

[72] Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 2007;26:731-43.

[73] Fazi F, Racanicchi S, Zardo G, Starnes LM, Mancini M, Travaglini L, et al. Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. Cancer Cell 2007;12:457-66.

[74] Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 2006;9:435-43.

[75] Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, et al. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. Cancer Cell 2015;27:286-97.

[76] Deshpande A, Pastore A, Deshpande AJ, Zimmermann Y, Hutter G, Weinkauf M, et al. 3'UTR mediated regulation of the cyclin D1 proto-oncogene. Cell Cycle 2009;8:3592-600.

[77] Liu Q, Fu H, Sun F, Zhang H, Tie Y, Zhu J, et al. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res 2008;36:5391-404.

[78] Yu Z, Baserga R, Chen L, Wang C, Lisanti MP, Pestell RG. microRNA, cell cycle, and human breast cancer. Am J Pathol 2010;176:1058-64.

[79] Kim YK, Yu J, Han TS, Park SY, Namkoong B, Kim DH, et al. Functional links between clustered microRNAs: suppression of cell-cycle inhibitors by microRNA clusters in gastric cancer. Nucleic Acids Res 2009;37:1672-81.

[80] Gillies JK, Lorimer IA. Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. Cell Cycle 2007;6:2005-9.

[81] Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafre SA, et al. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J Biol Chem 2007;282:23716-24.

[82] Visone R, Russo L, Pallante P, De Martino I, Ferraro A, Leone V, et al. MicroRNAs (miR)-221 and miR-222, both overexpressed in human thyroid papillary carcinomas, regulate p27Kip1 protein levels and cell cycle. Endocr Relat Cancer 2007;14:791-8.

[83] Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 2008;14:1271-7.

[84] Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC. miR-15a and miR-16-1 down-regulation in pituitary adenomas. J Cell Physiol 2005;204:280-5.

[85] Pichiorri F, Suh SS, Rocci A, De Luca L, Taccioli C, Santhanam R, et al. Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell 2010;18:367-81.

[86] Yan HL, Xue G, Mei Q, Wang YZ, Ding FX, Liu MF, et al. Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. EMBO J 2009;28:2719-32.

[87] Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol 2008;28:6773-84.

[88] Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593-601.

[89] Meng X, Wu J, Pan C, Wang H, Ying X, Zhou Y, et al. Genetic and epigenetic down-regulation of microRNA-212 promotes colorectal tumor metastasis via dysregulation of MnSOD. Gastroenterology 2013;145:426-36 e1-6.

[90] Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010;12:247-56.

[91] Ding X, Park SI, McCauley LK, Wang CY. Signaling between transforming growth factor beta (TGF-beta) and transcription factor SNAI2 represses expression of microRNA miR-203

to promote epithelial-mesenchymal transition and tumor metastasis. J Biol Chem 2013;288:10241-53.

[92] Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 2008;283:15878-83.

[93] Liu F, Lou YL, Wu J, Ruan QF, Xie A, Guo F, et al. Upregulation of microRNA-210 regulates renal angiogenesis mediated by activation of VEGF signaling pathway under ischemia/perfusion injury in vivo and in vitro. Kidney Blood Press Res 2012;35:182-91.

[94] Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol 2008;26:462-9.

[95] Stiegelbauer V, Perakis S, Deutsch A, Ling H, Gerger A, Pichler M. MicroRNAs as novel predictive biomarkers and therapeutic targets in colorectal cancer. World J Gastroenterol 2014;20:11727-35.

[96] Goh JN, Loo SY, Datta A, Siveen KS, Yap WN, Cai W, et al. microRNAs in breast cancer: regulatory roles governing the hallmarks of cancer. Biol Rev Camb Philos Soc 2016;91:409-28.

[97] Shen J, Todd NW, Zhang H, Yu L, Lingxiao X, Mei Y, et al. Plasma microRNAs as potential biomarkers for non-small-cell lung cancer. Lab Invest 2011;91:579-87.

[98] du Rieu MC, Torrisani J, Selves J, Al Saati T, Souque A, Dufresne M, et al. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. Clin Chem 2010;56:603-12.

[99] Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol 2007;8:R214.

[100] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005;353:1793-801.

[101] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006;9:189-98.

[102] Rothe F, Ignatiadis M, Chaboteaux C, Haibe-Kains B, Kheddoumi N, Majjaj S, et al. Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. PLoS One 2011;6:e20980.

[103] Toyama T, Kondo N, Endo Y, Sugiura H, Yoshimoto N, Iwasa M, et al. High expression of microRNA-210 is an independent factor indicating a poor prognosis in Japanese triple-negative breast cancer patients. Jpn J Clin Oncol 2012;42:256-63.

[104] Kasinski AL, Kelnar K, Stahlhut C, Orellana E, Zhao J, Shimer E, et al. A combinatorial microRNA therapeutics approach to suppressing non-small cell lung cancer. Oncogene 2015;34:3547-55.

[105] Zhang S, Shan C, Kong G, Du Y, Ye L, Zhang X. MicroRNA-520e suppresses growth of hepatoma cells by targeting the NF-kappaB-inducing kinase (NIK). Oncogene 2012;31:3607-20.

[106] He XX, Chang Y, Meng FY, Wang MY, Xie QH, Tang F, et al. MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. Oncogene 2012;31:3357-69.

[107] Sicard F, Gayral M, Lulka H, Buscail L, Cordelier P. Targeting miR-21 for the therapy of pancreatic cancer. Mol Ther 2013;21:986-94.

[108] Levin AA. Treating Disease at the RNA Level with Oligonucleotides. N Engl J Med 2019;380:57-70.

[109] Zhao Y, Deng C, Lu W, Xiao J, Ma D, Guo M, et al. let-7 microRNAs induce tamoxifen sensitivity by downregulation of estrogen receptor alpha signaling in breast cancer. Mol Med 2011;17:1233-41.

[110] Boyerinas B, Park SM, Murmann AE, Gwin K, Montag AG, Zillhardt M, et al. Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer 2012;130:1787-97.

[111] Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. Drug Metab Dispos 2009;37:2112-7.

[112] Bao L, Hazari S, Mehra S, Kaushal D, Moroz K, Dash S. Increased expression of P-glycoprotein and doxorubicin chemoresistance of metastatic breast cancer is regulated by miR-298. Am J Pathol 2012;180:2490-503.

[113] Valeri N, Gasparini P, Braconi C, Paone A, Lovat F, Fabbri M, et al. MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating human DNA MutS homolog 2 (hMSH2). Proc Natl Acad Sci U S A 2010;107:21098-103.

[114] Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 2008;68:425-33.

[115] Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology 2006;130:2113-29.

[116] Cittelly DM, Das PM, Salvo VA, Fonseca JP, Burow ME, Jones FE. Oncogenic HER2{Delta}16 suppresses miR-15a/16 and deregulates BCL-2 to promote endocrine resistance of breast tumors. Carcinogenesis 2010;31:2049-57.

[117] Bai WD, Ye XM, Zhang MY, Zhu HY, Xi WJ, Huang X, et al. MiR-200c suppresses TGF-beta signaling and counteracts trastuzumab resistance and metastasis by targeting ZNF217 and ZEB1 in breast cancer. Int J Cancer 2014;135:1356-68.

[118] Izumchenko E, Chang X, Michailidi C, Kagohara L, Ravi R, Paz K, et al. The TGFbeta-miR200-MIG6 pathway orchestrates the EMT-associated kinase switch that induces resistance to EGFR inhibitors. Cancer Res 2014;74:3995-4005.

[119] Mu W, Hu C, Zhang H, Qu Z, Cen J, Qiu Z, et al. miR-27b synergizes with anticancer drugs via p53 activation and CYP1B1 suppression. Cell Res 2015;25:477-95.

[120] Mittal A, Chitkara D, Behrman SW, Mahato RI. Efficacy of gemcitabine conjugated and miRNA-205 complexed micelles for treatment of advanced pancreatic cancer. Biomaterials 2014;35:7077-87.

[121] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.

[122] Jiang S, Zhang LF, Zhang HW, Hu S, Lu MH, Liang S, et al. A novel miR-155/miR-143 cascade controls glycolysis by regulating hexokinase 2 in breast cancer cells. EMBO J 2012;31:1985-98.

[123] Kim S, Rhee JK, Yoo HJ, Lee HJ, Lee EJ, Lee JW, et al. Bioinformatic and metabolomic analysis reveals miR-155 regulates thiamine level in breast cancer. Cancer Lett 2015;357:488-97.

[124] Eichner LJ, Perry MC, Dufour CR, Bertos N, Park M, St-Pierre J, et al. miR-378(*) mediates metabolic shift in breast cancer cells via the PGC-1beta/ERRgamma transcriptional pathway. Cell Metab 2010;12:352-61.

[125] Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 2009;458:762-5.

[126] Li B, He L, Zuo D, He W, Wang Y, Zhang Y, et al. Mutual Regulation of MiR-199a-5p and HIF-1alpha Modulates the Warburg Effect in Hepatocellular Carcinoma. J Cancer 2017;8:940-9.

[127] Peschiaroli A, Giacobbe A, Formosa A, Markert EK, Bongiorno-Borbone L, Levine AJ, et al. miR-143 regulates hexokinase 2 expression in cancer cells. Oncogene 2013;32:797-802.

[128] Gregersen LH, Jacobsen A, Frankel LB, Wen J, Krogh A, Lund AH. MicroRNA-143 down-regulates Hexokinase 2 in colon cancer cells. BMC Cancer 2012;12:232.

[129] Tanaka H, Sasayama T, Tanaka K, Nakamizo S, Nishihara M, Mizukawa K, et al. MicroRNA-183 upregulates HIF-1alpha by targeting isocitrate dehydrogenase 2 (IDH2) in glioma cells. J Neurooncol 2013;111:273-83.

[130] Puissegur MP, Mazure NM, Bertero T, Pradelli L, Grosso S, Robbe-Sermesant K, et al. miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. Cell Death Differ 2011;18:465-78.

[131] Jung KA, Lee S, Kwak MK. NFE2L2/NRF2 Activity Is Linked to Mitochondria and AMP-Activated Protein Kinase Signaling in Cancers Through miR-181c/Mitochondria-Encoded Cytochrome c Oxidase Regulation. Antioxid Redox Signal 2017;27:945-61.

[132] Aschrafi A, Schwechter AD, Mameza MG, Natera-Naranjo O, Gioio AE, Kaplan BB. MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons. J Neurosci 2008;28:12581-90.

[133] Subramanian S, Lui WO, Lee CH, Espinosa I, Nielsen TO, Heinrich MC, et al. MicroRNA expression signature of human sarcomas. Oncogene 2008;27:2015-26.

[134] Gits CM, van Kuijk PF, Jonkers MB, Boersma AW, van Ijcken WF, Wozniak A, et al. MiR-17-92 and miR-221/222 cluster members target KIT and ETV1 in human gastrointestinal stromal tumours. Br J Cancer 2013;109:1625-35.

[135] Haller F, von Heydebreck A, Zhang JD, Gunawan B, Langer C, Ramadori G, et al. Localization- and mutation-dependent microRNA (miRNA) expression signatures in gastrointestinal stromal tumours (GISTs), with a cluster of co-expressed miRNAs located at 14q32.31. J Pathol 2010;220:71-86.

[136] Choi HJ, Lee H, Kim H, Kwon JE, Kang HJ, You KT, et al. MicroRNA expression profile of gastrointestinal stromal tumors is distinguished by 14q loss and anatomic site. Int J Cancer 2010;126:1640-50.

[137] Yamamoto H, Kohashi K, Fujita A, Oda Y. Fascin-1 overexpression and miR-133b downregulation in the progression of gastrointestinal stromal tumor. Mod Pathol 2013;26:563-71.

[138] Akcakaya P, Caramuta S, Ahlen J, Ghaderi M, Berglund E, Ostman A, et al. microRNA expression signatures of gastrointestinal stromal tumours: associations with imatinib resistance and patient outcome. Br J Cancer 2014;111:2091-102.

[139] Ihle MA, Trautmann M, Kuenstlinger H, Huss S, Heydt C, Fassunke J, et al. miRNA-221 and miRNA-222 induce apoptosis via the KIT/AKT signalling pathway in gastrointestinal stromal tumours. Mol Oncol 2015;9:1421-33.

[140] Koelz M, Lense J, Wrba F, Scheffler M, Dienes HP, Odenthal M. Down-regulation of miR-221 and miR-222 correlates with pronounced Kit expression in gastrointestinal stromal tumors. Int J Oncol 2011;38:503-11.

[141] Kim WK, Park M, Kim YK, Tae YK, Yang HK, Lee JM, et al. MicroRNA-494 downregulates KIT and inhibits gastrointestinal stromal tumor cell proliferation. Clin Cancer Res 2011;17:7584-94.

[142] Yun S, Kim WK, Kwon Y, Jang M, Bauer S, Kim H. Survivin is a novel transcription regulator of KIT and is downregulated by miRNA-494 in gastrointestinal stromal tumors. Int J Cancer 2018;142:2080-93.

[143] Shi Y, Gao X, Hu Q, Li X, Xu J, Lu S, et al. PIK3C2A is a gene-specific target of microRNA-518a-5p in imatinib mesylate-resistant gastrointestinal stromal tumor. Lab Invest 2016;96:652-60.

[144] Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, et al. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. Cancer Res 2012;72:1126-36.

[145] Niinuma T, Kai M, Kitajima H, Yamamoto E, Harada T, Maruyama R, et al. Downregulation of miR-186 is associated with metastatic recurrence of gastrointestinal stromal tumors. Oncol Lett 2017;14:5703-10.

[146] Tu L, Wang M, Zhao WY, Zhang ZZ, Tang DF, Zhang YQ, et al. miRNA-218-loaded carboxymethyl chitosan - Tocopherol nanoparticle to suppress the proliferation of gastrointestinal stromal tumor growth. Mater Sci Eng C Mater Biol Appl 2017;72:177-84.

[147] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029-33.

[148] Warburg O, Wind F, Negelein E. The Metabolism of Tumors in the Body. J Gen Physiol 1927;8:519-30.

[149] Cardaci S, Zheng L, MacKay G, van den Broek NJ, MacKenzie ED, Nixon C, et al. Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. Nat Cell Biol 2015;17:1317-26.

[150] Kim SY. Targeting cancer energy metabolism: a potential systemic cure for cancer. Arch Pharm Res 2019;42:140-9.

[151] Warburg O. On the origin of cancer cells. Science 1956;123:309-14.

[152] Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 2011;10:671-84.

[153] Israelsen WJ, Dayton TL, Davidson SM, Fiske BP, Hosios AM, Bellinger G, et al. PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. Cell 2013;155:397-409.

[154] Vyas S, Zaganjor E, Haigis MC. Mitochondria and Cancer. Cell 2016;166:555-66.

[155] Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci U S A 2010;107:8788-93.

[156] Lussey-Lepoutre C, Hollinshead KE, Ludwig C, Menara M, Morin A, Castro-Vega LJ, et al. Loss of succinate dehydrogenase activity results in dependency on pyruvate carboxylation for cellular anabolism. Nat Commun 2015;6:8784.

[157] Sullivan LB, Martinez-Garcia E, Nguyen H, Mullen AR, Dufour E, Sudarshan S, et al. The proto-oncometabolite fumarate binds glutathione to amplify ROS-dependent signaling. Mol Cell 2013;51:236-48.

[158] Zhang J, Pavlova NN, Thompson CB. Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. EMBO J 2017;36:1302-15.

[159] Mayers JR, Wu C, Clish CB, Kraft P, Torrence ME, Fiske BP, et al. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. Nat Med 2014;20:1193-8.

[160] Lanning NJ, Looyenga BD, Kauffman AL, Niemi NM, Sudderth J, DeBerardinis RJ, et al. A mitochondrial RNAi screen defines cellular bioenergetic determinants and identifies an adenylate kinase as a key regulator of ATP levels. Cell Rep 2014;7:907-17.

[161] Kamphorst JJ, Cross JR, Fan J, de Stanchina E, Mathew R, White EP, et al. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. Proc Natl Acad Sci U S A 2013;110:8882-7.

[162] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-93.

[163] Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 2011;25:460-70.

[164] Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, et al. Oncogenes and signal transduction. Cell 1991;64:281-302.

[165] Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. Science 2013;342:1242454.

[166] Ito K, Suda T. Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol 2014;15:243-56.

[167] Dimitrakopoulou-Strauss A, Ronellenfitsch U, Cheng C, Pan L, Sachpekidis C, Hohenberger P, et al. Imaging therapy response of gastrointestinal stromal tumors (GIST) with FDG PET, CT and MRI: a systematic review. Clin Transl Imaging 2017;5:183-97.

[168] Vitiello GA, Medina BD, Zeng S, Bowler TG, Zhang JQ, Loo JK, et al. Mitochondrial Inhibition Augments the Efficacy of Imatinib by Resetting the Metabolic Phenotype of Gastrointestinal Stromal Tumor. Clin Cancer Res 2018;24:972-84.

[169] Huang WK, Gao J, Chen Z, Shi H, Yuan J, Cui HL, et al. Heterogeneity of Metabolic Vulnerability in Imatinib -Resistant Gastrointestinal Stromal Tumor. Cells 2020;9.

[170] Chekulayev V, Mado K, Shevchuk I, Koit A, Kaldma A, Klepinin A, et al. Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes. Biochem Biophys Rep 2015;4:111-25.

[171] Schell JC, Olson KA, Jiang L, Hawkins AJ, Van Vranken JG, Xie J, et al. A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. Mol Cell 2014;56:400-13.

[172] Vellinga TT, Borovski T, de Boer VC, Fatrai S, van Schelven S, Trumpi K, et al. SIRT1/PGC1alpha-Dependent Increase in Oxidative Phosphorylation Supports Chemotherapy Resistance of Colon Cancer. Clin Cancer Res 2015;21:2870-9.

[173] Denise C, Paoli P, Calvani M, Taddei ML, Giannoni E, Kopetz S, et al. 5-fluorouracil resistant colon cancer cells are addicted to OXPHOS to survive and enhance stem-like traits. Oncotarget 2015;6:41706-21.

[174] Rebane-Klemm E, Truu L, Reinsalu L, Puurand M, Shevchuk I, Chekulayev V, et al. Mitochondrial Respiration in KRAS and BRAF Mutated Colorectal Tumors and Polyps. Cancers (Basel) 2020;12.

[175] Kamo N, Naomoto Y, Shirakawa Y, Yamatsuji T, Hirota S, Fujiwara Y, et al. Involvement of focal adhesion kinase in the progression and prognosis of gastrointestinal stromal tumors. Hum Pathol 2009;40:1643-9.